Washer Dispenser

EL406™

Operator's Manual

BioTek®

EL406™ Microplate Washer Dispenser Operator's Manual

BioTek Instruments, Inc.
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Notices

BioTek® Instruments, Inc.

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Document Conventions

This manual uses the following typographic conventions:

- This note format calls attention to important information.

Warnings are presented in this style to call attention to potential hazards and other safety concerns.

This icon calls attention to important safety information.

Tips and suggestions for improving performance are formatted this way.

- **Water**: Daily maintenance is the key to keeping the liquid handler performing to specifications. In the maintenance procedures provided in this manual, the requirement to use distilled (dH2O) or deionized (DI) water can be met by numerous water purification methods, including MilliQ™. A minimum water purity of 2mOhm is expected.

Revision History

Rev	Date	Changes
A	10/2008	First Issue
B	4/2009	Full keypad control was added to EL406 and documented in this manual. Chapter 3, Operations includes complete instructions for operating the instrument using the keypad.
		1536-well plate processing capability is available with required hardware. Operating instructions and physical descriptions were updated where applicable throughout the manual.
		New 1536-well hardware includes 128-tube aspirate manifold and two 32-tube Syringe dispenser manifolds. Installation instructions, Specifications, operation and maintenance sections were updated accordingly.
		AutoPrime functionality was changed:

Rev	Date	Changes
		• Submerge tips, also known as soaking, capability was added; • First-use control to trigger function was removed. AutoPrime, when enabled, always runs regardless of whether the device has been used since startup.
C	5/2010	Added content where applicable throughout the manual to support new hardware options: single 96-tube wash manifold, and dual 8-tube Syringe dispenser manifold.
		Noted the absence of some features on certain models where applicable, i.e. the Peri-pump, Buffer Switching, and Ultrasonic Advantage are no longer standard on all models.
		Added description of new keypad feature to manually control the vacuum pump: Shift+1/2 in the Quick menus.
		Maintenance: Removed warning about predefined maintenance protocols for those using the BioStack under keypad control. The EL406 no longer delivers plates during prime routines and maintenance protocols.
D	10/2010	Content throughout this manual was changed, where applicable, to correspond to the new basecode, PN 7180207. This manual no longer supports previously released instruments with basecode PN 7180200. Changes include the keypad main menu and other menus.
		Added content where applicable to describe two new accessory kits, Vacuum Filtration and Syringe Dispenser Buffer Switching
		Where applicable, content about the LHC was revised to describe new behavior and procedures for LHC 2.
E	9/2012	Added support for 0.5 µL dispensing. Added more precise instructions for handling 1536-well flanged plates (153F). Added warning about the effects of exposing silicone tubing to DMSO and Acetonitrile to the chemical compatibility table. Revised the Syringe dispenser performance specifications to correct for minor mismatches with BioTek's published specifications. Defined the water purity expectations for maintenance procedures and changed all references to "DI water" to "deionized or distilled water." Updated IVD notice.

Intended Use Statement

- The EL406™ Microplate Washer Dispenser provides microplate priming, washing, and dispensing for ELISA™, fluorescence and chemiluminescence immunoassays, cellular and agglutination assays.

- The EL406™ Microplate Washer Dispenser can operate as a stand-alone instrument or with standard robotic systems, such as BioTek's BioStack™ Microplate Stacker.
- If the instrument has an "IVD" label it may be used for clinical and non-clinical purposes, including research and development. If there is no such label the instrument may only be used for research and development and non-clinical purposes.

Quality Control

It is considered good laboratory practice to run laboratory samples according to instructions and specific recommendations included in the assay package insert for the test to be conducted. Failure to conduct Quality Control checks could result in erroneous test data.

Warranty and Product Registration

Please take a moment to review the Warranty information that shipped with your product. Please also register your product with BioTek to ensure that you receive important information and updates about the product(s) you have purchased.

You can register online through BioTek's Customer Resource Center (CRC) at www.biotek.com or by calling 888/451-5171 or 802/655-4740.

Repackaging and Shipping

If you need to ship the instrument to BioTek for service or repair, contact BioTek for a Return Materials Authorization (RMA) number and use the original packing materials. Other forms of commercially available packaging are not recommended and can void the warranty. If the original packing materials have been damaged or lost, contact BioTek for replacement packing.

Warnings

Use the instrument on a level, stable surface away from excessive humidity. When operated in a safe environment, according to the instructions in this manual, there are no known hazards associated with the EL406. However, the operator should be aware of certain situations that could result in serious injury: Do not reach into the instrument during operation, as the washer manifold or peristaltic pump (Peri-pump) pump barrel may pinch your fingers. Do not reach for the microplate carrier until it is in its home position.

Strict adherence to instrument maintenance and qualification procedures is required to ensure accurate dispense volumes and risk-free operation.

Hazards and Precautions

Hazards

The following hazards are provided to help avoid injury:

Warning! Power Rating. The instrument's power supply or power cord must be connected to a power receptacle that provides voltage and current within the specified rating for the system. Use of an incompatible power receptacle may produce electrical shock and fire hazards.

Warning! Electrical Grounding. Never use a two-prong plug adapter to connect primary power to the external power supply. Use of a two-prong adapter disconnects the utility ground, creating a severe shock hazard. Always connect the power cord directly to an appropriate receptacle with a functional ground.

Warning! Internal Voltage. Always turn off the power switch and unplug the power supply before cleaning the outer surface of the instrument or removing its top case.

Warning! Liquids. Avoid spilling liquids on the instrument; fluid seepage into internal components creates a potential for shock hazard or instrument damage. If a spill occurs while a program is running, abort the program and turn the instrument off. Wipe up all spills immediately. Do not operate the instrument if internal components have been exposed to fluid.

Warning! Potential Biohazards. Some assays or specimens may pose a biohazard. Adequate safety precautions should be taken as outlined in the assay's package insert. This hazard is noted by the symbol shown here. Always wear safety glasses and appropriate protective equipment, such as chemically resistant rubber gloves and apron.

Warning! Unspecified Use. Failure to operate this equipment according to the guidelines and safeguards specified in this manual could result in a hazardous condition.

Warning! Ultrasonic Energy. Ultrasonic energy is present in the ultrasonic cleaner reservoir (if equipped) when AUTOCLEAN programs are running. Avoid putting your fingers in the bath. Ultrasonic energy can be destructive to human tissue.

Warning! Pinch Hazard. Some areas of the instrument or its components can present pinch hazards when the instrument is operating. These areas are marked with the symbol shown here. Keep hands/fingers clear of these areas when the instrument is operating.

Warning! Software Quality Control. The operator must follow the

manufacturer's assay package insert when modifying software parameters and establishing reading, washing, or dispensing methods. **Failure to conduct quality control checks could result in erroneous test data.**

Warning! Service. Only qualified technical personnel should perform service procedures on internal components.

Warning! Accessories. Only accessories which meet the manufacturer's specifications shall be used with the instrument.

Precautions

The following precautions are provided to help avoid damage to the instrument:

Caution: Service. The instrument should be serviced by BioTek authorized service personnel. Only qualified technical personnel should perform troubleshooting and service procedures on internal components.

Caution: Environmental Conditions. Do not expose the instrument to temperature extremes. For proper operation, ambient temperatures should remain within the range listed in the ***Specifications*** section. Performance may be adversely affected if temperatures fluctuate above or below this range. Storage temperature limits are broader.

Caution: Sodium Hypochlorite. Do not expose any part of the instrument to the recommended diluted sodium hypochlorite solution (bleach) for more than 20 minutes. Prolonged contact may damage the instrument surfaces. Be certain to rinse and thoroughly wipe all surfaces.

Caution: Buffer Solution. Although many precautions have been taken to ensure that the instrument is as corrosion-proof as possible, the instrument is not sealed and liquids can seep into sensitive components. Make sure that any spilled buffer solution is wiped off the instrument. Prolonged exposure to salt solution may corrode parts of the microplate carrier, movement rail, springs, and other hardware.

Caution: Chemical Compatibility. Some chemicals may cause irreparable damage to the instrument. The following chemicals have been deemed safe for use in the instrument: buffer solutions (such as PBS), saline, surfactants, deionized water, 70% ethyl, isopropyl, or methyl alcohol, 40% formaldehyde, and 20% sodium hydroxide. Never use acetic acid, DMSO, or other organic solvents. These chemicals may cause severe damage to the instrument. Contact BioTek prior to using other questionable chemicals.

Caution: Bovine Serum Albumin. Solutions containing proteins, such as bovine serum albumin (BSA), will compromise the instrument's performance over time unless a strict maintenance protocol is adhered to. See ***Maintenance*** procedures regarding BSA.

Caution: External Power Supply. Only use the power supply shipped with the instrument. Operate this power supply within the range of line voltages

listed on it.

Caution: Disposal. This instrument contains printed circuit boards and wiring with lead solder. Dispose of the instrument according to Directive 2002/96/EC, "on waste electrical and electronic equipment (WEEE)," or local ordinances.

Caution: Warranty. Failure to follow preventive maintenance protocols may **void the warranty.**

Caution: Shipping Hardware. All shipping hardware (e.g., shipping bracket etc.) must be removed before operating the instrument and reinstalled before repackaging the instrument for shipment.

Caution: High Flow Pump Installation. DO NOT plug the High Flow vacuum pump cable into a wall outlet! Use the adapter provided with the pump to connect the pump to the accessory outlet on the back of the washer. See the ***Installation*** instructions*.*

Caution: Waste Sensor Port on EL406. (For customers who have purchased the BioStack Microplate Stacker.) Although the waste sensor port on the back of the EL406 is the same type as the 24-VDC power connector on the back of the BioStack, if an external 24-VDC power supply is plugged into the EL406's port, **it will permanently damage internal components**.

Caution: Do not run the Peri-pump without a cassette installed on the pump.

Caution: Electromagnetic Environment. Per IEC 61326-2-6 it is the user's responsibility to ensure that a compatible electromagnetic environment for this instrument is provided and maintained in order that the device will perform as intended.

Caution: Electromagnetic Compatibility. Do not use this device in close proximity to sources of strong electromagnetic radiation (e.g., unshielded intentional RF sources), because these may interfere with the proper operation.

Caution: Spare Parts. Only approved spare parts should be used for maintenance. The use of unapproved spare parts and accessories may result in a loss of warranty and potentially impair instrument performance or cause damage to the instrument.

CE Mark

CE	Based on the testing described below and information contained herein, this instrument bears the CE mark.

▪ **Note:** See the Declaration of Conformity for specific information.

Directive 2004/108/EC: Electromagnetic Compatibility

Emissions—Class A

The system has been type-tested by an independent, accredited testing laboratory and found to meet the requirements of EN 61326-1: Class A for Radiated Emissions and Line Conducted Emissions.

Verification of compliance was conducted to the limits and methods of EN 55011 (CISPR 11) Class A. In a domestic environment it may cause radio interference, in which case, you may need to mitigate the interference.

Immunity

The system has been type-tested by an independent, accredited testing laboratory and found to meet the requirements of EN 61326-1 and EN 61326-2-6 for Immunity. Verification of compliance was conducted to the limits and methods of the following:

EN 61000-4-2, Electrostatic Discharge

EN 61000-4-3, Radiated EM Fields

EN 61000-4-4, Electrical Fast Transient/Burst

EN 61000-4-5, Surge Immunity

EN 61000-4-6, Conducted Disturbances from RFI

EN 61000-4-8 Power Frequency Magnetic Field Immunity Test

EN 61000-4-11, Voltage Dips, Short Interruptions and Variations

Directive 2006/95/EC Low Voltage (Safety)

The system has been type-tested by an independent testing laboratory and was found to meet the requirements of this Directive. Verification of compliance was conducted to the limits and methods of the following:

EN 61010-1, “Safety requirement for electrical equipment for measurement, control and laboratory use. Part 1, General requirements.”

Directive 2002/96/EC: Waste Electrical and Electronic Equipment

Disposal Notice: This instrument contains printed circuit boards and wiring with lead solder. Dispose of the instrument according to Directive 2002/96/EC, “on waste electrical and electronic equipment (WEEE)” or local ordinances.

Directive 2002/95/EC: Reduction of Hazardous Substances (RoHS)

This instrument is exempt from RoHS requirement per Article 2, Category 8.

Directive 98/79/EC: In Vitro Diagnostics (if labeled for this use)

- Product registration with competent authorities.
- Traceability to the U.S. National Institute of Standards and Technology (NIST).

EN 61010-2-101 Particular requirements for in vitro diagnostic (IVD) medical equipment.

Electromagnetic Interference and Susceptibility

USA FCC CLASS A

RADIO AND TELEVISION INTERFERENCE

NOTE: This equipment has been tested and found to comply with the limits for a Class A digital device, pursuant to Part 15 of the FCC Rules. These limits are designed to provide reasonable protection against harmful interference when the equipment is operated in a commercial environment. This equipment generates, uses, and can radiate radio frequency energy and, if not installed and used in accordance with the instruction manual, may cause harmful interference to radio communications. Operation of this equipment in a residential area is likely to cause harmful interference, in which case the user will be required to correct the interference at their own expense.

In order to maintain compliance with FCC regulations shielded cables must be used with this equipment. Operation with non-approved equipment or unshielded cables is likely to result in interference to radio and television reception.

Canadian Department of Communications Class A

This digital apparatus does not exceed Class A limits for radio emissions from digital apparatus set out in the Radio Interference Regulations of the Canadian Department of Communications.

Le present appareil numerique n'émet pas de bruits radioelectriques depassant les limites applicables aux appareils numerique de la Class A prescrites dans

le Reglement sur le brouillage radioelectrique edicte par le ministere des Communications du Canada.

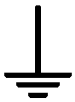
User Safety

This device has been type-tested by an independent laboratory and found to meet the requirements of the following:

- **Underwriters Laboratories UL 61010-1** "Safety requirements for electrical equipment for measurement, control and laboratory use; Part 1: general requirements."
- **Canadian Standards Association CAN/CSA C22.2 No. 61010-1** "Safety requirements for electrical equipment for measurement, control and laboratory use; Part 1: general requirements."
- EN 61010 Standards, see CE Mark on page xv.

Safety Symbols

Some of these symbols appear on the instrument or accessories:

	Alternating current Courant alternatif Wechselstrom Corrientealterna Correntealternata		Both direct and alternating current Courant continu et courant alternatif Gleich - und Wechselstrom Corriente continua y corrientealterna Corrente continua e correntealternata
	Direct current Courant continu Gleichstrom Corriente continua Corrente continua		Earth ground terminal Borne de terre Erde (Betriebserde) Borne de tierra Terra (difunzionamento)
	On (Supply) Marche (alimentation) Ein (VerbindungmitdemNetz) Conectado Chiuso		Protective conductor terminal Borne de terre de protection Schutzleiteranschluss Borne de tierra de protección Terra diprotezione
	Off (Supply) Arrêt (alimentation) Aus (TrennungvomNetz) Desconectado Aperto (scon-nessionedallaretedialimentazione)		Caution (refer to accompanying documents) Attention (voir documents d'accompanement) AchtungsieheBegleitpapiere Atención (vease los documentosincluidos) Attenzione, consultare la doc annessa
	Warning, risk of electric shock Attention, risque de choc électrique Gefährlicheelektrischeschlag Precaución, riesgo de sacudidaeléctrica Attenzione, rischiodiscossaelettrica		Warning, risk of crushing or pinching Attention, risqued'écrasement et pincement Warnen, Gefahr des Zerquetschens und Klemmen Precaución, riesgo del machacamiento y sejeción Attenzione, rischiodischiacciareedintrappolarsi
	Warning, hot surface Attention, surface chaude Warnen, heißeOberfläche Precaución, superficiecaliente Attenzione, superficiecalda		Warning, potential biohazards Attention, risquesbiologiquespotentiels Warnung! MoeglichebiologischeGiftstoffe Atención, riesgosbiológicos Attenzione, rischiobiologico
	In vitro diagnostic medical device Dispositif médical de diagnostic in vitro Medizinisches In-Vitro-Diagnostikum Dispositivo médico de diagnóstico in		Separate collection for electrical and electronic equipment Les équipements électriques et électroniques font l'objet d'une collecte sélective

	vitro Dispositivo medico diagnostico in vitro		Getrennte Sammlung von Elektro- und Elektronikgeräten Recogida selectiva de aparatos eléctricos y electrónicos Raccolta separata delle apparecchiature elettriche ed elettroniche
	Consult instructions for use Consulter la notice d'emploi Gebrauchsanweisung beachten Consultar las instrucciones de uso Consultare le istruzioni per uso		

Chapter 1

Introduction

Thank you for purchasing the EL406™ Microplate Washer Dispenser. This chapter describes the instrument's features and specifications and includes important contact information.

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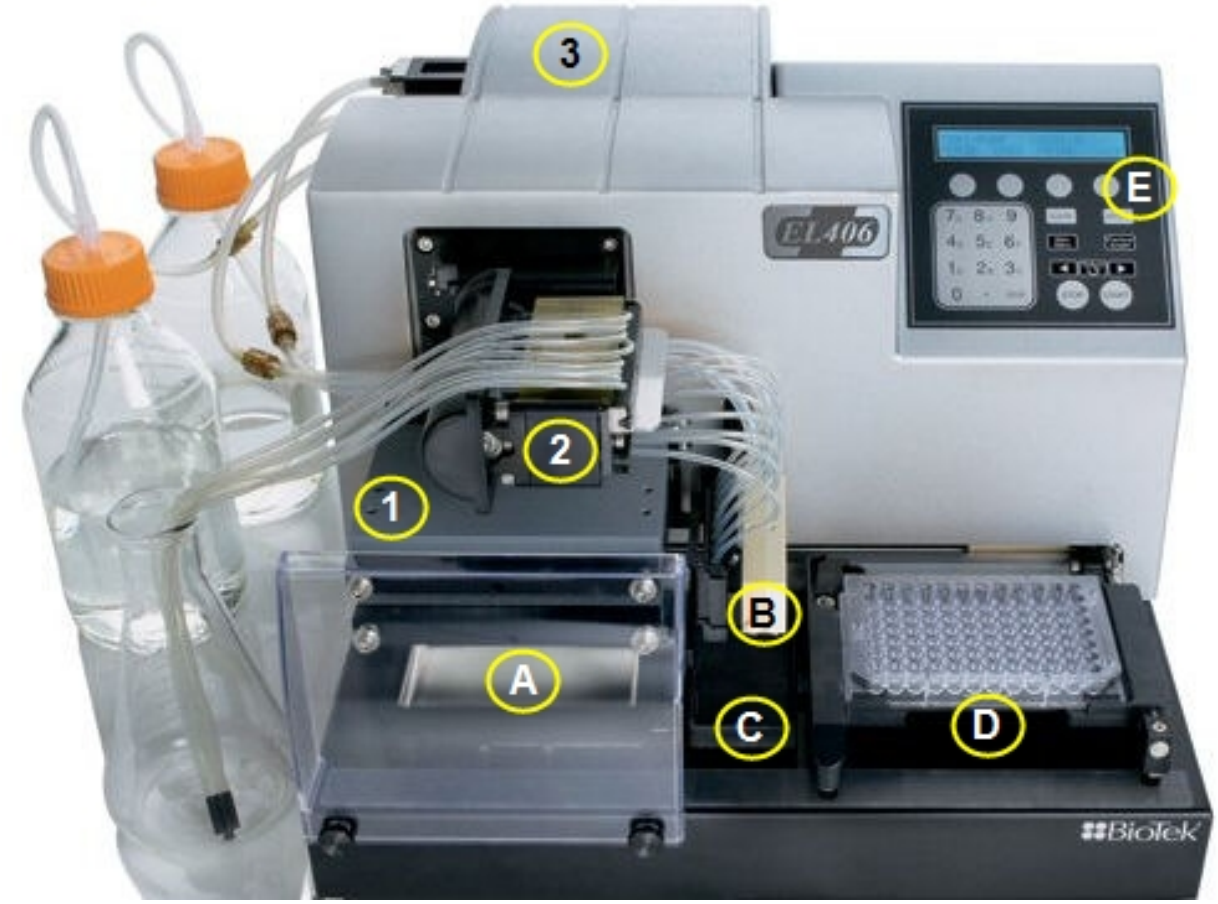
Introducing the EL406™ Washer Dispenser

The EL406 offers up to three devices in one instrument: a microplate Washer, a peristaltic pump dispenser called the Peri-pump, and a dual Syringe pump dispenser. One or both dispensers are provided with every EL406.



	Device/Component	Description
1	Washer manifold	96- or 192-tube devices aspirate and dispense fluid; or 128-tube manifold aspirates fluid from 1536-well plates.
2	Peri-pump Dispenser	Peristaltic, 8-channel dispenser with entirely visible fluid path.
3	(Optional) Syringe Dispenser	Two distinct syringe-pump dispensers, each with an 8-, 16- or 32-tube manifold.
A	Ultrasonic cleaning and priming trough	Most models support AutoClean ultrasonic cleaning of the washer.

	Device/Component	Description
B	Dispense arm	Holds the Peri-pump's Tip Holder and the Syringe dispensers' manifolds.
C	Priming trough	Waste reservoir for collecting priming fluid for the Peri-pump and Syringe dispensers.
D	Plate Carrier	Holds standard microplates for processing.
E	Keypad	One of two ways to control the instrument; the other way is LHC.

- The valve on the left side of some instrument models (not shown in photograph) is for the optional Vacuum Filtration Accessory Kit.

Features of the EL406

- Supports all microplate-based assays, including ELISA, fluorescence, chemiluminescence, RIA, DNA probes, and cellular assays.
- A variety of solutions, including buffered saline and reagents can be dispensed.
- The intuitive onboard software allows you to create and store up to 99 wash and dispense protocols. BioTek provides numerous predefined protocols for maintenance and instrument qualification purposes.
- Compatible with BioTek's BioStack™ Microplate Stacker for automated plate processing.
- A robot-accessible carrier that can be interfaced into some robotic systems.
- Computer control using BioTek's **Liquid Handling Control™software** ("LHC").
- A low-maintenance design, the result of BioTek's long history with liquid-handling instruments.

Washer

- Programmable dispense volumes, and a wide range of wash options, from gentle washing for cellular assays to vigorous washing for ELISA™.
- A "bottom washing" routine to lower the background absorbance and "crosswise" or secondary aspiration to reduce residual volumes, except in 1536-well plates.
- Supports Wash, Prime, Dispense, and Aspirate steps (except dispensing to 1536-well plates, which is performed with the dispensers).
- An optional buffer-switching module with four supply bottles supports complex assays by allowing one protocol to automatically draw reagent from up to four distinct reservoirs.

- Built-in fluid, flow, and vacuum detection provide complete confidence for unattended operation.
- Several predefined protocols are provided to simplify preventative maintenance, which should be performed regularly to ensure optimum washer performance.
- BioTek's patent-pending Ultrasonic Advantage™ (ultrasonic cleaner) provides extra cleaning power by using ultrasonic pulses in a water bath to remove residue on the manifold tubes. A stainless steel cleaning reservoir with an ultrasonic transducer bonded to the bottom of the reservoir is mounted on the washer. An AutoClean function enables the user to run ultrasonic cleaning routines.

Peri-pump Dispenser

- A peristaltic pump with eight individual tubes transfers fluid from a supply bottle, or up to eight different supply bottles, to various vessels. The pump has four rollers over which the tubing is stretched.
- The tubing is contained in an easy to load and unload cassette that is attached to the pump head. The pump's protective cover must be in place to run a dispense routine.
- Three cassette sizes are available: 1 µL, 5 µL, and 10 µL for the most precise dispensing of volumes from 1 to 3000 µL and 0.5 µL dispenses with certain models using a 1 µL cassette.
- Autoclavable tubing (steam temperatures and pressures of 121° C and 1 bar (750 mmHg)) is compatible with 70% ethyl or isopropyl alcohol and 0.5% sodium hypochlorite (bleach) solution for easy maintenance.

Syringe Dispensers

- The Syringe dispenser has a long-lasting seal that ensures precise and accurate fluid delivery, as well as reproducibility for repeated dispenses.
- Two syringes support distinct fluid sources:
    - 16-channel: one tube per well for 384-well plates and two tubes per well for 96-well plates.
    - 32-channel: one tube per well for 1536-well plates,
    - 8-channel (two manifolds in one block): one tube per well for both 96- and 384-well plates.
- Autoclavable components can be used with organic solvents and provide easy maintenance.
- Does not require recalibration.

Liquid Handling Control™ (LHC) Software

BioTek's Liquid Handling Control (LHC) software lets you control the instrument from your computer. You'll enjoy the convenience of programming assay-specific wash and dispense protocols in a familiar Windows environment (Microsoft® Windows® 7, Vista, and Windows XP).

For high-throughput applications, the LHC supports BioStack™ integration.

Please refer to the LHC Installation Guide and Help system to learn about:

- Installing the LHC software on the controlling computer
- Running Maintenance protocols
- Running Qualification protocols
- Special considerations when operating with the BioStack Microplate Stacker

EL406 Dispenser Comparison

Counting the washer as a potential dispenser, the EL406 offers three distinct dispensers to choose from. Here is a comparison of the devices:

- For **precious reagents** use the Peri-pump to preserve unused fluids. It has the shortest, most visible fluid path, and a Purge capability to reverse the fluid flow and recover fluid from the tubing. Another advantage is the ability to dedicate usage of a dispense cassette to one reagent only, reducing the amount of priming required prior to use.

<table>
<tr><th>Device</th><th>Volume range µL/well</th><th>Precision</th><th>Accuracy</th><th>Approximate Dead volume</th></tr>
<tr><td>Peri-pump</td><td>0.5‡, and 1-3000*</td><td><10% CV @ 1 µL/well (typical <3% CV)</td><td>+/-10% (typical +/-3%) @ 1 µL/well</td><td>Cassette Type:<table><tr><td>1 µL</td><td>1.20 mL</td></tr><tr><td>5 µL</td><td>4.23 mL</td></tr><tr><td>10 µL</td><td>7.36 mL</td></tr></table></td></tr>
<tr><td>Syringe 8-tube</td><td>10-3000</td><td><5% CV @ 20 µL/well</td><td>±2 µL @ 10 µL/well</td><td rowspan="3">For all manifold types: 12 mL without Buffer Switching. The Buffer Switching module adds 5 mL per fluid path.</td></tr>
<tr><td>Syringe 16-tube</td><td>5-3000</td><td><10% CV @ 5 µL/well</td><td>±2 µL @ 10 µL/well</td></tr>
<tr><td>Syringe 32-tube</td><td>3-3000</td><td><12% CV @ 6 µL/well</td><td>±5% @ 6 µL/well</td></tr>
<tr><td>Washer Dual/Single 96-tube</td><td>50-3000</td><td><3.0% CV @ 300 µL/well</td><td>300 µL: ±5%</td><td rowspan="2">125 mL without Buffer Switching. The Buffer Switching module adds 45 mL to the fluid path.</td></tr>
<tr><td>Washer 192-tube</td><td>25-5000</td><td><4.0% CV @ 80 µL/well</td><td>80 µL: ±5%</td></tr>
</table>

*1 µL cassettes' maximum recommended dispense volume is 50 µL/well.
‡ 0.5 µL dispensing is supported by some late-model instruments using a 1 µL cassette.

BioTek recommends priming a dispenser with three times its dead volume to prepare it for accurate dispensing.

Processing Time §

Protocols were optimized for speed to obtain the following processing times, including the fastest flow and travel rates. Some of these parameters are listed in the Parameters column of the table.

Device	Plate Type	Volume (µL/well)	Parameters	Time in seconds ¥
Peri-pump - 5 µL	96	10	High flow rate	3
Peri-pump - 1 µL	384	1	High flow rate	6
	1536	1	High flow rate	21
Syringe 8-tube	96	20	Flow rate 1	6.5
Syringe 16-tube	96	20	Flow rate 1	5.25
	384	20	Flow rate 1	14
Syringe 32-tube	1536	3	One SB manifold	16.5
	1536	14	Both SB manifolds	27
Washer Dual/Single 96-tube	96	300	3 cycles	< 30
Washer Dual 96-tube	384	100	3 cycles	< 80
Washer 192-tube	384	400	1 cycle	< 20
1536 Wash: 128-aspirate tubes, 32-dispense tubes	1536	10	1 cycle, Plate Clearance = 90; Both SB manifolds.	< 32
	1536	10	1 cycle, Plate Clearance = 60; one SB manifold.	< 46

§ Review the **Specifications** for more details.

¥ Excluding plate carrier and manifold homing movements.

SB = small bore Syringe manifold

BioStack Compatibility

The EL406 is compatible with BioTek's BioStack Microplate Stacker. The BioStack can rapidly transfer microplates one-at-a-time to and from the instrument, and includes:

- Removable stacks (one input and one output).
- Optional restacking of plates to maintain correct sequencing.
- The ability to continue processing plates following the aborting/failure of one plate.
- The ability to pause processing to allow the user to add more plates to the input stack or to remove some from the output stack.

If you have purchased the BioStack to operate with the EL406, refer to the BioStack Operator's Manual for instructions on configuring the EL406 to run with the BioStack. To help you get started: **See Operating with the BioStack on page 82**.

If you are interested in purchasing the BioStack, contact your local BioTek dealer for more information or visit our website at www.biotek.com.

Package Contents

- Part numbers and package contents are subject to change and vary according to instrument model. Please contact BioTek Customer Care if you have any questions.

Description	PN
Power cord (part numbers vary by country of use)	Varies
RS-232 serial cable	75034
USB cable (USB Virtual COM Port Driver Software & instructions)	75108
Microplate carrier	7180501
Priming trough insert (1) for Peri-pump	7182043
Priming trough insert (2) for Syringe dispensers	7182044
Mist shield and thumbscrews (2) ~ *installed*	7182042
Strip plate (12x1)	98265
Screwdriver, Phillips	98268
Stylus: for cleaning washer manifold aspirate tubes	7102108
Stylus: for cleaning washer and Syringe manifold dispense tubes and 128-tube aspirate manifold tubes	2872304
Stylus: for cleaning 192-tube dispense manifold	7102139
10 cc syringe and tubing for Peri-pump cassette maintenance	49919
Shipping brackets (2)	7182005 7182073
Hex wrench: 9/64”	48434
Hex wrench: 3/32” for removing syringe pumps	48570
Hex wrench: 1/16” for removing magnets from syringe dispense manifolds	48713
Spare fuses (5)	46055
EL406™ Getting Started Guide (and Operator’s Manual on CD - PN 7181009)	7181010

- Some components are model specific, they ship only with certain instrument models.

Optional Accessories

- Part numbers and package contents are subject to change and vary according to instrument model. Please contact BioTek Customer Care if you have any questions.

General Instrument Accessories

Description		PN
BioTek liquid testing solutions for instrument qualification tests	Wetting Agent	7773002
	Blue Test Dye	7773001
Large size priming trough insert		7182109
Liquid Handling Control™ Software		LHC2
BioStack™ Microplate Stacker and integration kit		Biostack
Installation-Operational-Performance Qualification (IQ-OQ-PQ) package		7180527

Washer Accessories

Description		PN
Complete dispense/waste system, 4 Liter bottles	115 volts	7100547
	230 volts	7100548
Complete dispense/waste system with High Flow vacuum pump (recommended for use with 384-well microplates), 4L bottles	115 volts	7100565
	230 volts	7100566
Auxiliary power cord for vacuum pump connection		75096
Vacuum tubing set		7100533
Dispense tubing set		7100538
Buffer Switching module with 4 supply bottles		7100540

Description		PN
10-liter dispense bottle		7100559
Waste bottles	10 liter	7100557
	20 liter	7100556
Vacuum pump standard	115 volts	7100562
	230 volts	7100561
High Flow vacuum pump: used when washing 384-well microplates with buffers not containing surfactants or where strong aspiration is required	115 volts	7100563
	230 volts	7100564

Magnetic Bead Assay Accessories

Accessory	PN
Magnet Adapter Kit	7180011
Magnets:	
384-well Flat Magnet	7103017
384-well Ring Magnet	7102215
96-well Flat Magnet	7103016
96-well Ring Magnet	7102216

Vacuum Filtration Kit

Description	PN
Vacuum Filtration Kit	7180029
-- 96-well Only	1170008
-- 96- and 384-well	1170009

Peri-pump Optional Accessories

- Part numbers and package contents are subject to change and vary according to instrument model. Please contact BioTek Customer Care if you have any questions.

Dispense cassettes and accessories:

Cassette Type	Cassette	Tips	Replacement tubing kit*	Tubing extension kit
1 µL plastic tips	7170012	7172150	7170009	7170022
1 µL 1536-well, plastic tips	7170018	7172150		
1 µL sapphire jeweled stainless steel tips*	7170015	48692		
1 µL 1536-well, sapphire jeweled stainless steel tips*	7170016	48692		
5 µL plastic tips	7170011	7172059	7170008	7170021
5 µL stainless steel tips	7170014	7172128		
5 µL plastic, large bore tips	7170024	7172039		
10 µL plastic tips	7170010	7172059	7170007	7170020
10 µL stainless steel tips	7170013	7172128		
10 µL plastic, large bore tips	7170024	7172039		

*Save your stainless steel tips for reuse with a replacement kit, they ship with plastic tips.

Accessory	PN
Cassette Calibration Kit	7170017
Peri-pump Reservoir Holder	7210509
40 mL Priming Trough Insert	7182109

Syringe Dispenser Optional Accessories

- Part numbers and package contents are subject to change and vary according to instrument model. Please contact BioTek Customer Care if you have any questions.

Accessory	PN
Autoclavable Syringe Dispenser Module: 16-Tube Manifold (2)	7180006S
Buffer Switching Module	7180028
Stylus – for cleaning 8-/16-tube dispense manifold tubes	2872304
Stylus – for cleaning 32-tube LB dispense manifold tubes	7182095
Stylus – for cleaning 32-tube SB dispense manifold tubes	7182102

Accessory	PN
Inline Filters (2)	48705
Spare tubing sets (2 - 1/dispenser), autoclavable	7183006
DMSO- & Acetonitrile-safe tubing sets (2 - 1/dispenser)	7183002
Special large-bore 8-tube manifold for 96-well plates	7180549S

Physical Specifications

Labware	
Microplates	96-well, 384-well, and 1536-well that comply with SBS microplate standards 1-2004, 2-2004, 3-2004, and 4-2004.
Microstrips	1 x 8, 1 x 12
Microwells	Flat, round, "V" bottom

Hardware & Environmental	
User Interface	2-line x 24 character LCD screen, 26 alphanumeric soft keys
Power Supply	The instrument uses two internal power supplies: 24-volt 60 watt and 48-volt 60 watt. These supplies are compatible with 100-240 V~; 50-60 Hz.
Accessory Outlet	≤ 5.0 A, used for vacuum pump
Dimensions (W x D x H)	16½ x 12 5/8 x 18 inches (42 cm x 32 cm x 46 cm)
Weight (≤)	32 lb (14.5 kg)
Operating Conditions	10° - 40°C (50° - 104°F)
Relative Humidity	The instrument should be operated in a non-condensing humid environment having a maximum relative humidity of 80% at temperatures up to 31°C decreasing linearly to 50% relative humidity at 40°C.

Washer

Manifold Type	
96-tube	Single or Dual manifold with 96 sets of aspirate and dispense tubes arranged in an 8x12 array. Single manifolds can only process 96-well microplates; dual manifolds can process 96- and 384-well plates.
192-tube	Dual manifold with 192 sets of aspirate and dispense tubes arranged in a 16 x 12 array can only process 384-well plates.
128-tube	Aspirate-only manifold with 128-tubes in 32 x 4 array to process 1536-well plates. One dispense pin in the manifold fills the priming trough for maintenance procedures.

Waste bottle volume	4, 10, or 20 liters, depending on the accessory package, (2 bottles, one with sensor)
Supply bottle volume	2 4L or 10L bottles (4 bottles w/ Buffer Switching)

Peri-Pump

Peristaltic pump: Positive-displacement peristaltic pump with 4 rollers that stretch the 8 tubes (one per channel) to deliver fluid.

Cassette Types	Dispense range	Cassette Life	Dead Volume
1 µL	0.5, 1 - 3000 µL	1000 384-well plates @ 5 µL/well	1.2 mL
5 µL	5 - 3000 µL	1000 96-well plates @ 50 µL/well	4.2 mL
10 µL	10 - 3000 µL	1000 96-well plates @ 100 µL/well	7.4 mL

Syringe Dispenser

Two external positive-displacement syringe pump dispensers which support various manifold types.

Manifold Type	
8-Tube	2 x 8-channel non-autoclavable manifold with replaceable stainless steel tubes to process 96- and 384-well plates.
16-Tube	1 x 16-channel autoclavable manifold with replaceable stainless steel tubes to process 96- and 384-well plates.
16-Tube 7°	1 x 16-channel autoclavable manifold with replaceable stainless steel tubes to process 96- and 384-well plates. Tubes are angled 7 degrees

Manifold Type	
	to minimize turbulence in the wells when dispensing.
32-Tube	1 x 32-channel manifold cannot be autoclaved, and does not support non-factory tube replacement. An inline 90-micron filter is included to minimize clogs. For 1536-well plates only.

Performance Specifications

Washer

Average Residual Volume (Evacuation Efficiency)	
96-Tube Manifold (Single and Dual)	Average residual volume in the microwells is ≤ 2 µL per well after a 3-cycle wash, when 300 µL of deionized water with 0.1% Tween 20®, or buffer equivalent, is dispensed per well into a Costar® 96-well flat-bottom plate. The aspirate height adjustment is optimized for the plate prior to testing.
192-Tube Manifold	Average residual volume in the microwells is ≤2 µL per well after a 3-cycle wash, when 100 µL of deionized water with 0.1% Tween 20, or buffer equivalent, is dispensed per well into a Costar 384-well flat-bottom plate. The aspirate height adjustment is optimized for the plate prior to testing.
128-Tube Manifold	Average residual volume in the wells shall be ≤ 0.1 µL per well when 10 µL of deionized water with 0.1% Tween 20 solution is dispensed per well into a 1536-well flat-bottom Nunc® plate.

Vacuum Filtration Evacuation Efficiency	
96-Well Filter Plates	Average increased weight of the plate is ≤ 1.2 grams after dispensing 300 µL of deionized water per well into a Millipore® MSHVN4450 96-well 0.45µm plates (PN 98258) and vacuum aspirated for 30 seconds at vacuum pressure 2.5 Hg (+/- 0.5 Hg) and blotted on a paper towel.
384-Well Filter Plates	Average increased weight of the plate is ≤ 4.0 grams after dispensing 80 µL of deionized water per well into a Millipore® MZFCN0W10 384-well 1.2µm plates (PN 98287) and vacuum aspirated for 10 seconds at vacuum pressure 2.5 Hg (+/- 0.5 Hg) and blotted on a paper towel.

Dispense Accuracy	
96-Tube Manifold	± 5% when dispensing 300 µL per well of deionized water with 0.1% Tween 20, with FD&C #1 blue dye at a rate of 300 µL per well per second into a Costar 96-well flat-bottomed plate. The weight of the fluid dispensed shall be measured gravimetrically.
192-Tube Manifold	±5% when dispensing 80 µL per well of deionized water with 0.1% Tween 20, with FD&C #1 blue dye at a rate of 102 µL per well per second into a Costar 384-well flat-bottomed plate. The weight of the fluid dispensed shall be measured gravimetrically.

Dispense Precision	
96-Tube Manifold	≤ 3.0% CV when dispensing 300 µL per well of deionized water with 0.1% Tween 20, with FD&C #1 blue dye at a rate of 300 µL per well per second into a Costar 96-well flat-bottomed plate. The absorbance of the solution is read at 630 nm and 450 nm reference.
192-Tube Manifold	≤ 4.0% CV when dispensing 80 µL per well of deionized water with 0.1% Tween 20, with FD&C #1 blue dye at a rate of 102 µL per well per second into a Costar 384-well flat-bottomed plate. The absorbance of the solution is read at 630 nm and 450 nm reference.

Peri-Pump Dispenser

Precision is measured for a whole 96-well or 384-well plate using room-temperature deionized or distilled water with 0.1% Tween 20 with FD&C #1 blue dye. Precision is measured for 1536-well plates by dispensing to 384 wells, 12 columns with a 15% isopropyl alcohol solution. The absorbance of the solution is read at 630 nm and 450 nm reference. Specifications apply to volumes that are full unit increments for the cassette to which they apply, except the 1 µL cassette also supports 0.5 µL increments when dispensing this volume. For example: the precision specification for a 10 µL cassette is valid at 10, 20, 30, ..., 3000 µL; the 1 µL cassette precision specification is valid at 0.5, 1, 2, 3, ..., 3000 µL.

Accuracy is measured gravimetrically when dispensing room-temperature deionized water. Specifications apply to volumes that are full unit increments for the cassette to which they apply. For example: the accuracy specification for a 10 µL cassette is valid at 10, 20, 30, ... 3000 µL.

Cassette	Precision	Accuracy
1 µL	10%CV @ 1 µL per well	± 10% @ 1 µL per well
	5%CV @ 2 µL per well*	± 5% @ 2 µL per well*
	10%CV @ 0.5 µL per well	n/a
5 µL	5%CV @ 5 µL per well	± 4% @ 5 µL per well
	2.5%CV @ 10 µL per well*	± 2% @ 10 µL per well*
10 µL	4%CV @ 10 µL per well	± 4% @ 10 µL per well
	2%CV @ 20 µL per well*	± 2% @ 20 µL per well*
* These specifications are for these dispense volumes and higher.		

Cassette Expected Lifetime

Cassette Types	Cassette Life	Total Volume
1 µL	1000 384-well plates @ 5 µL/well	2,000 mL
5 µL	1000 96-well plates @ 50 µL/well	5,000 mL
10 µL	1000 96-well plates @ 100 µL/well	10,000 mL

With strict adherence to best practices and maintenance recommendations, this is the typical longevity of the dispense cassettes.

Syringe Dispensers

Precision is measured for a whole 96-well or 384-well plate using room-temperature deionized or distilled water with 0.1% Tween 20 with FD&C #1 blue dye. Precision is measured for 1536-well plates by dispensing to 384 wells, 12 columns with a 15% isopropyl alcohol solution. The absorbance of the solution shall be read at 630 nm and 450 nm reference.

Accuracy is measured gravimetrically when dispensing room-temperature deionized water.

Dispense Precision	
8-Tube:	≤ 2% CV when dispensing 100 µL/well ≤ 5% CV precision at 20 µL/well ≤ 5% CV precision at 40 µL/well ** ** Tested in-house to <4.0% CV.
16-Tube:	≤ 2% CV when dispensing 100 µL/well ≤ 2.5% CV precision at 80 µL/well*** ≤ 5% CV precision at 20 µL/well ≤ 10% CV precision at 5 µL/well* *unspecified for non-autoclavable syringe pumps. *** Tested in-house to <1.6% CV.
32-Tube:	< 12% CV when dispensing 6 µL per tube

Dispense Accuracy	
8-Tube	For all volumes 2 µL or 1%, whichever is greater, at flow rate 2.
16-Tube:	For all volumes 2 µL or 1%, whichever is greater, at flow rate 2.
32-Tube:	± 5% when dispensing 6 µL/well at flow rate 3.

BioTek's Customer Resource Center

BioTek's Customer Resource Center (CRC) continues our tradition of superior service and support. After an easy registration process, you can access lots of useful information about your BioTek microplate instrumentation and software. On the secure CRC website, you can:

- Track orders
- Access warranty information, user manuals and software updates
- Download technical and application information
- Maintain equipment inventory (product registration)
- Request service and technical support
- View service history
- And much more!

Register at https://customer.biotek.com

- Dispense cassette data sheets are available for download at the CRC.

Chapter 2

Installation

This chapter provides detailed installation instructions.

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Unpack and Inspect the Instrument

Important: Save all packaging materials. If you need to ship the instrument or accessories to BioTek for repair or replacement, you must use the original packaging. Using other forms of commercially available packaging is not recommended and can void the warranty. Improper packaging that results in damage to the instrument may lead to additional charges. Refer to the operator's manual for repacking instructions.

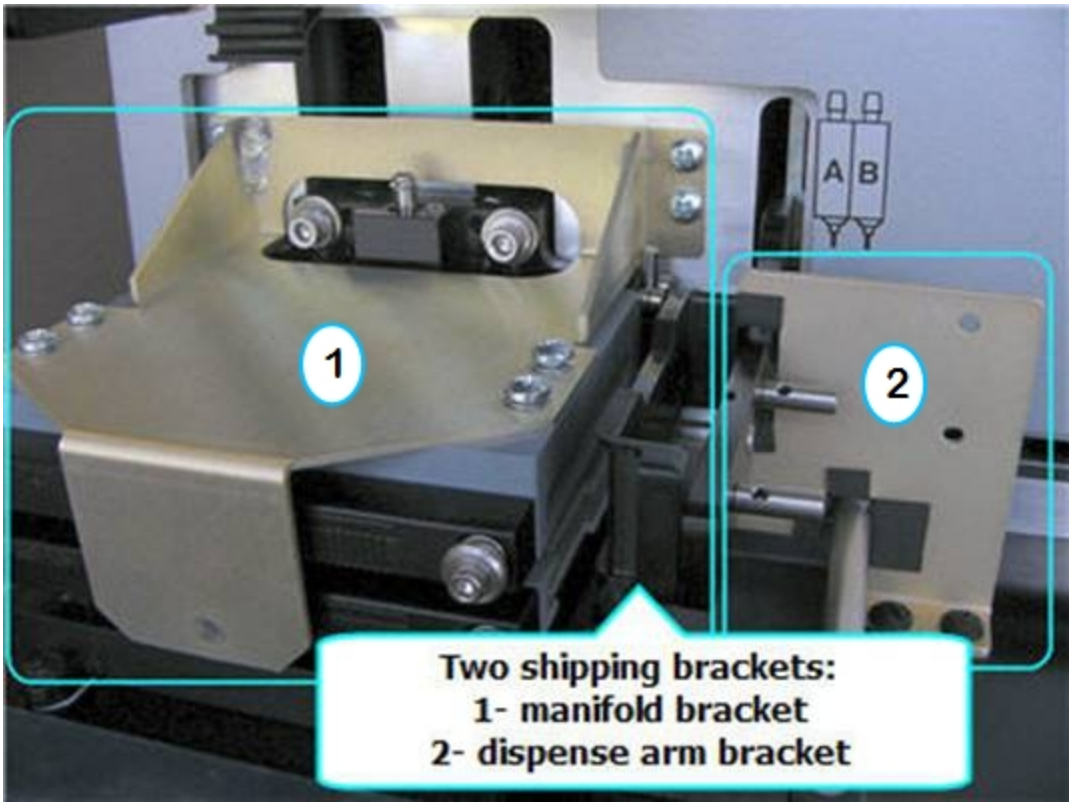
Inspect the shipping box, packaging, instrument, and accessories for signs of damage.

If the EL406™ Microplate Washer Dispenser is damaged, notify the carrier and your BioTek representative. Keep the shipping cartons and packing material for the carrier's inspection. BioTek will arrange for repair or replacement of your instrument immediately, before the shipping-related claim is settled.

1. Unpack the boxes containing the instrument and other equipment:
   - EL406™ Microplate Washer Dispenser and accessories
   - Vacuum Pump and accessories
   - Buffer switching valve module and accessories
   - Vacuum Filtration Accessory Kit
   - Dual Syringe Dispenser and accessories
   - Additional 192-tube washer manifolds for 384-well plate processing or 128-tube wash and 32-tube dispense manifolds for 1536-well plate processing.
2. Place all packing materials back into the shipping boxes for reuse if necessary.

Refer to the Package Contents on page 9 to make sure you have all expected equipment.

Remove the Shipping Hardware



Supplies

A Phillips-head screwdriver is needed to remove the shipping brackets.

Keep in mind that you will have to reinstall the shipping hardware and use the original shipping material if it is necessary to return the instrument to BioTek for service or repair.

Remove the rubber band

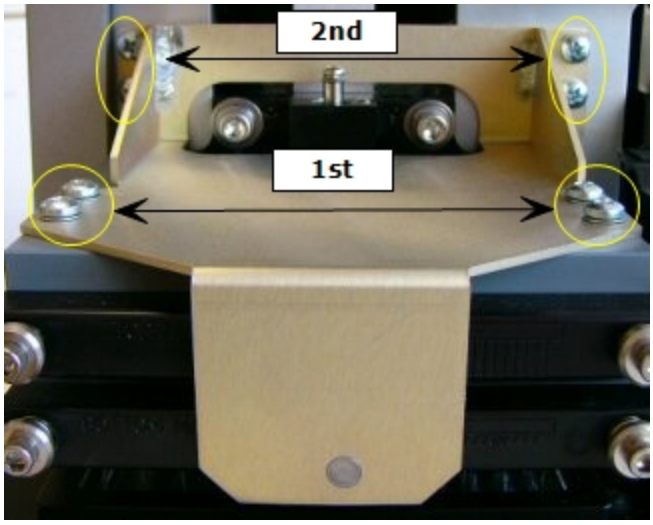
1. Carefully remove the rubber band that holds the pump cover in place around the **Peri-pump**, if present.

Store the rubber band in the small plastic storage pouch for future use.

Attach the plastic storage pouch to the rear panel

2. Use the Velcro strips to attach the plastic storage pouch to the rear panel of the instrument. Select a position that does not block the vent holes.

Remove the manifold shipping bracket



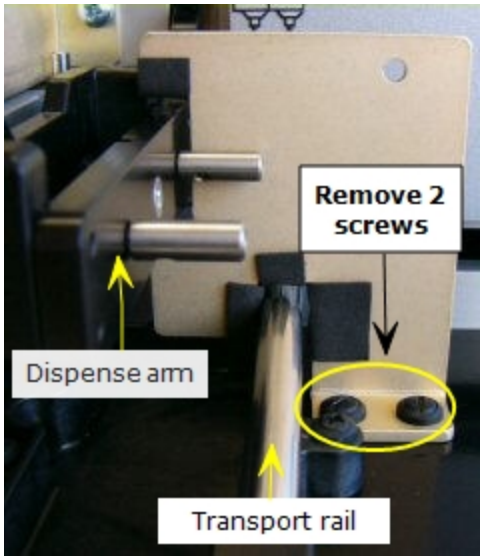
1. Use the Phillips screwdriver to remove the
   8 screws and washers holding the bracket in place. First remove the 4 screws from the manifold, then remove the screws from the instrument.
2. Slide the bracket towards you and remove.
3. Store the bracket, screws and washers with the other shipping material.

- Single 96-tube manifolds have two spacers on top of the manifold under the shipping bracket, to make it fit. Be sure to save these spacers with the shipping materials or in the pouch on the back of the instrument. They must be reinstalled prior to shipping.

Remove the dispense arm shipping bracket

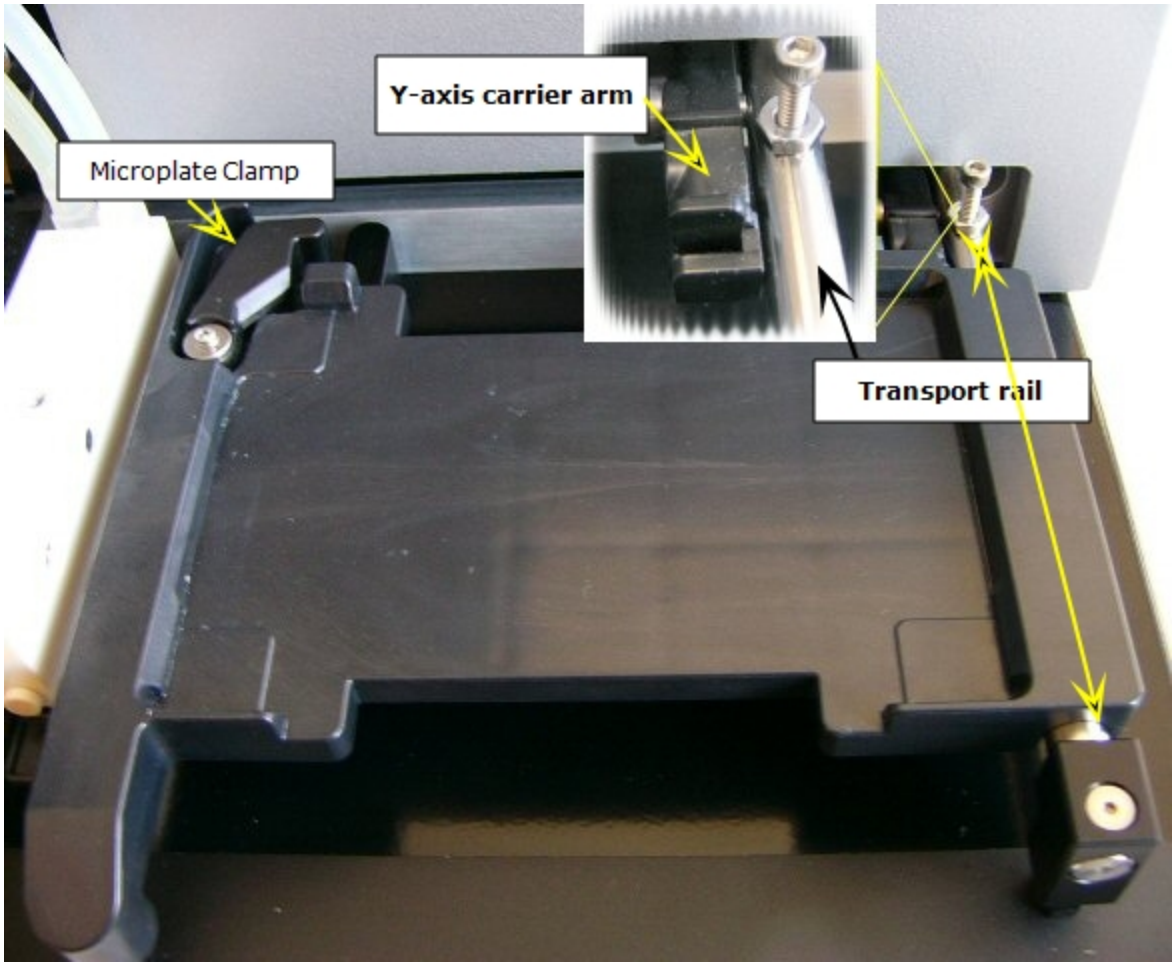
The bracket holds in place both the dispense arm and plate carrier rail during shipping.

1. Use the screwdriver to remove the 2 screws holding the bracket in place.
2. Gently lift the dispense arm slightly to release the bracket and remove it.
3. Reinsert the screws into the instrument's base plate for safe keeping.
4. Store the bracket with the other shipping material.



Set Up the Washer

Install the Microplate Carrier



The **Y-axis carrier arm** attached to the **Transport rail** holds the plate carrier. It moves the plate during processing to allow the dispensers to address high density plates.

1. Locate the microplate carrier in the accessories box.
2. Slide the plate carrier transport rail all the way to the right, and then back to the left about 1-2 inches (3-4 cm). The transport rail was being held in place by the dispense-arm shipping bracket.
3. Look at the underside of the carrier to visualize how it fits in place: its two legs will be positioned on the left and the curved hollow fits over the transport rail on the right. Note the small slit adjacent to the top of the curved hollow. This slit fits into the **Y-axis Carrier Arm**.

4. Aligning the curved hollow with the transport rail, place the carrier on the rail so it fits into the slot on the **Y-axis Carrier Arm**. If necessary, release the spring-loaded microplate clamp in the back left corner of the carrier to level the carrier on the instrument.

- If a 401 Instrument Error is displayed at startup, recheck the plate carrier position. Make sure the plate carrier is seated in the **Y-axis Carrier Arm** next to the transport rail.

Setting Up the EL406

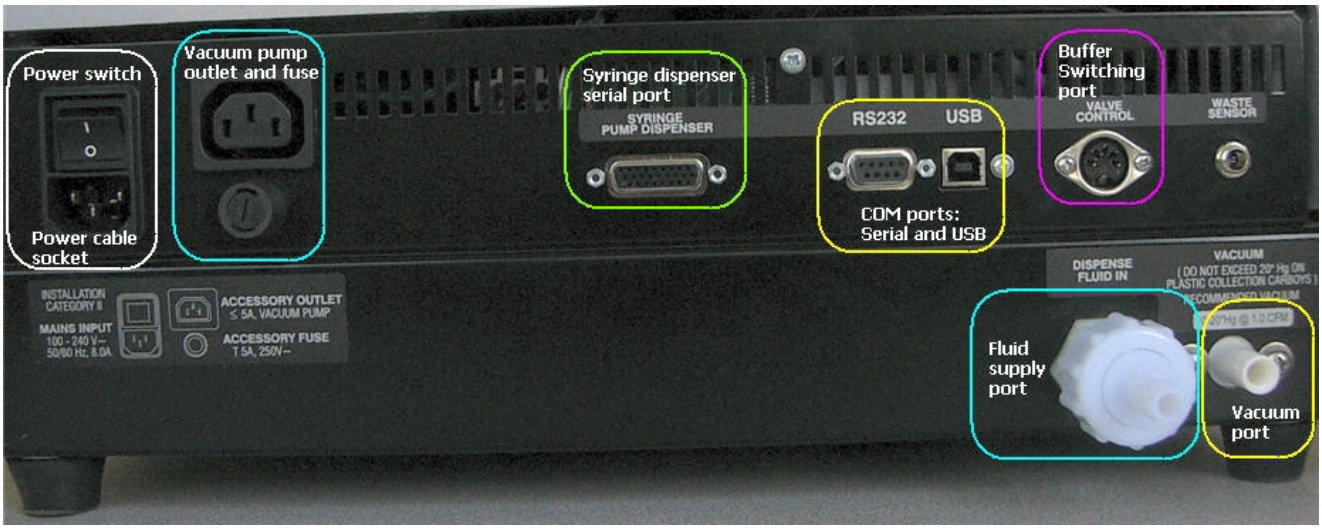
Important: Avoid **excessive humidity.** Condensation directly on the sensitive electronic circuits can cause the instrument to fail internal self checks.

Install the instrument on a level, stable surface in an area where ambient temperatures between 10°C (50°F) and 40°C (104°F) can be maintained.

The instrument should be operated in a non-condensing humid environment having a maximum relative humidity of 80% at temperatures up to 31°C decreasing linearly to 50% relative humidity at 40°C.

Connect the Vacuum Pump, Tubes, and Bottles

For optimal operation of the EL406, all tubing, cables, and fittings for the fluid supply and waste systems must be properly connected. This image illustrates the rear panel of the instrument and the locations of the ports and connections for the fluid supply and waste systems.



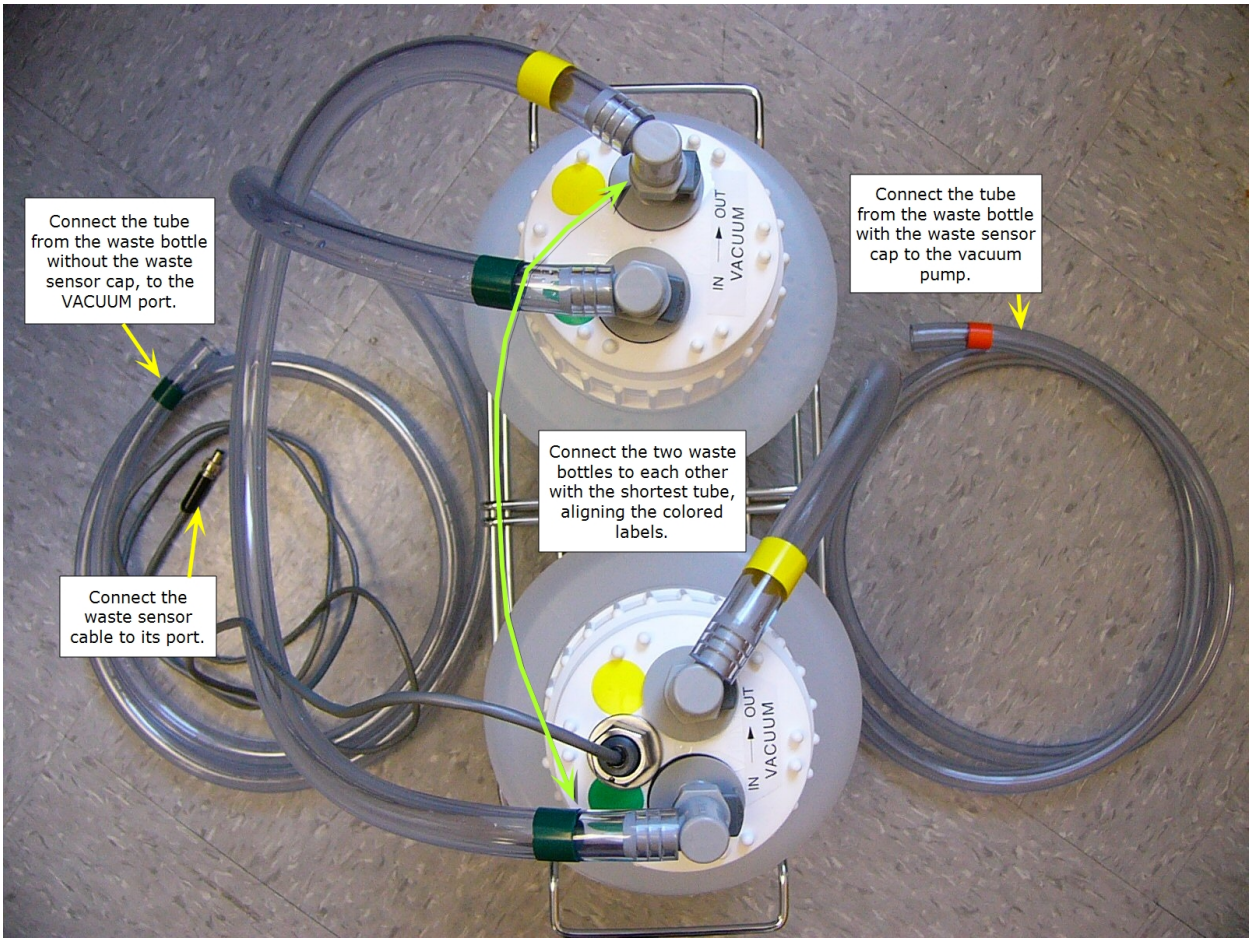
Rear Panel

Waste System

Caution! Pump Installation. Do not plug the vacuum pump cable into a wall outlet! Use the adapter provided with the pump to connect it to the **Accessory Outlet** on the back of the instrument. This allows the EL406 to regulate the pump, turning it on and off as specified by the protocol.

When using a standard pump (rather than the high flow pump), set the instrument's **Vacuum Dissipation Delay** to prevent the pump from drawing excess current and blowing the 5-amp fuse. **See Define Instrument Settings on page 51**

- **Note:** The waste tubes have colored bands that match similarly colored dots next to the inlet/outlet ports on the waste bottle caps to ensure the correct connection of the tubing.



Waste System

Three lengths of tubing are shipped with the waste module:

Tubing:		Connects:
Short tube with yellow and green bands	→	The two waste bottles to each other
Long tube with green bands on both ends	→	Bottle without sensor to vacuum **port**
Long tube with yellow and orange bands	→	Bottle with waste sensor to the vacuum **pump**

1. Locate the quick-release caps shipped inside the waste bottles and attach the tubing to them as follows:

2. Connect the waste bottles to each other using the shortest length of tubing, matching the colored bands on the tubing to colored dots on the caps.
3. Attach the waste sensor cable to the **Waste Sensor** port on the back of the washer.
4. Attach the tube from the **waste bottle with the waste sensor** in its cap to the vacuum pump.
5. Attach the tube from the **waste bottle that does NOT have the waste sensor** in its cap to the **Vacuum** port on the back of the instrument.
6. **Important!** When installing BioTek's vacuum pump, connect the pump's AC power cable to the vacuum pump **Accessory Outlet** on the back of the instrument (Use the accessory outlet adapter provided, if applicable.)
7. Place the waste bottles and vacuum pump on the same horizontal plane as the instrument or below it, such as the floor beneath the work surface. This will help optimize performance.
8. Make sure the waste bottle's caps are well sealed.

- BioTek strongly recommends installing the vacuum line filter to protect your vacuum pump.

Install the Vacuum Line Filter

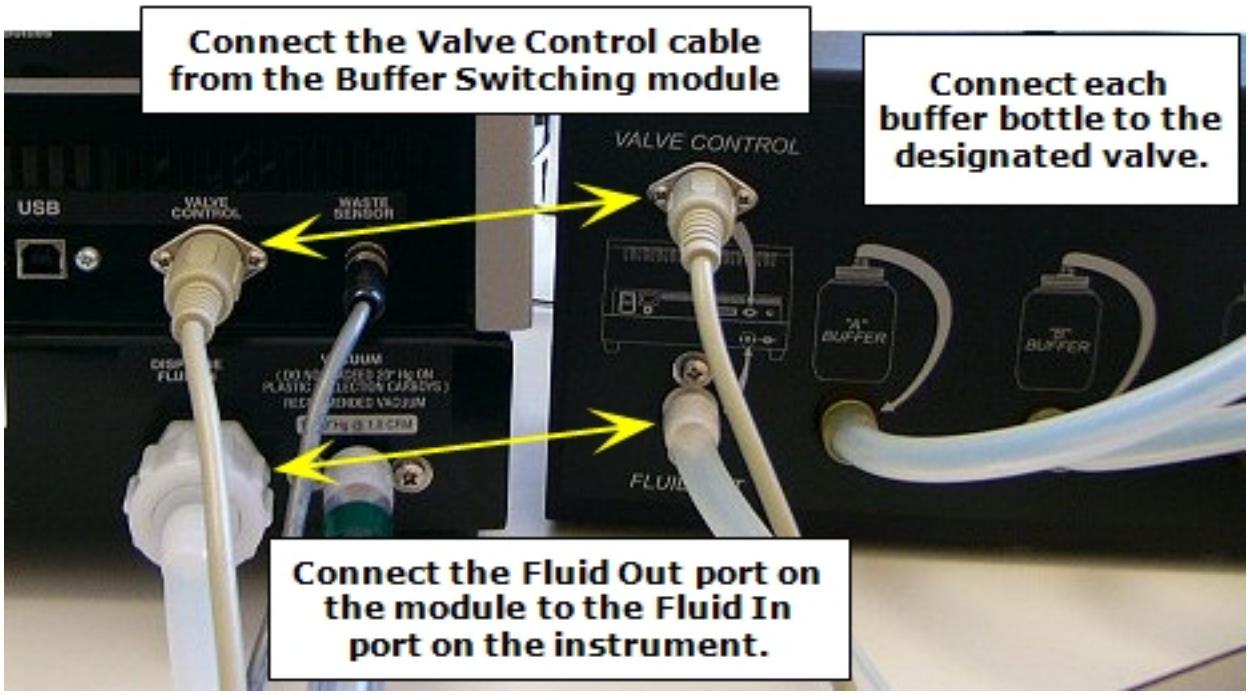
The optional vacuum line filter (PN 48294) can be installed halfway between the last waste bottle (overflow bottle) and the vacuum pump.

To do this, cut the tubing and insert the filter, noting the direction of flow. The flow arrow on the filter should point **toward the vacuum pump**.

In the event of a fluid overflow, the filter should prevent the destruction of the vacuum pump's internal components. If an overflow does occur, check the filter for trapped fluid. If fluid is found in the filter, remove the filter and drain using the small white nut on top of the filter. Tighten the white nut and reinstall the filter.

Fluid Supply System

Install the Buffer Switching module



Buffering Switching System

1. Place the four supply bottles and valve module on the same surface as the instrument to optimize performance.
2. Connect the cable from the Valve Control port on the module to the Valve Control port on the back of the instrument.
3. Connect the tubing from one of the supply bottles to "A" Buffer in the valve module.
4. Repeat step 3 with the other three supply bottles for "B," "C," and "D" Buffers.
5. Connect the 6-foot (1.83 Meter) tubing from the valve box Fluid Out port to the Dispense Fluid In port on the instrument's rear panel. This tubing can be cut to the optimal length required for the installation.

Important: Instruments that specify Buffer Switching ("B" models) are configured at the factory to use the module to supply fluid. If you choose not to use the module and instead connect a supply bottle directly to the instrument, be sure to change the washer's buffer switching setting: Change the Washer Buffer Switching Setting on page 160.

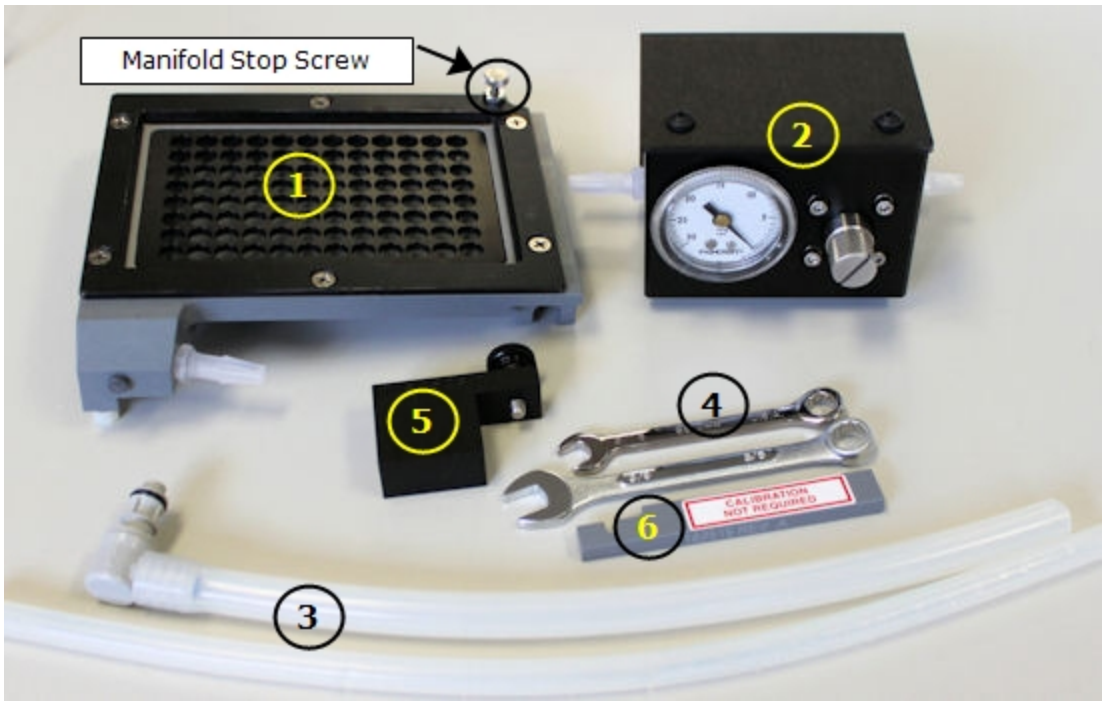
- **Important**: Only one buffer switching unit can be installed at a time, either the washer's buffer switching module or the Syringe's. Thus, the instrument's settings for these devices work like a toggle, when one is enabled, the other is disabled, if applicable.

Install the Fluid Supply without Buffer Switching

1. There is one supply tube. Connect one end to the Dispense Fluid In port and the other end to the supply bottle.
2. Place the supply bottle on the same horizontal plane as the instrument.

Set up the Vacuum Filtration Accessory

If applicable, complete these steps to prepare the instrument to perform vacuum filtration assays. Otherwise, skip this step.



The Vacuum Filtration Accessory Kit includes:

1. Special vacuum filtration plate carrier
2. Pressure regulator
3. Evacuation tubing
4. Two wrenches: 3/8” (10 mm) and 5/16" (7.94 mm)
5. Foot Adjustment tool for measuring plate carrier leveling feet
6. Adjustment tool for positioning the manifold stop screw

You must provide a standard microplate with a cover.

To set up vacuum filtration:

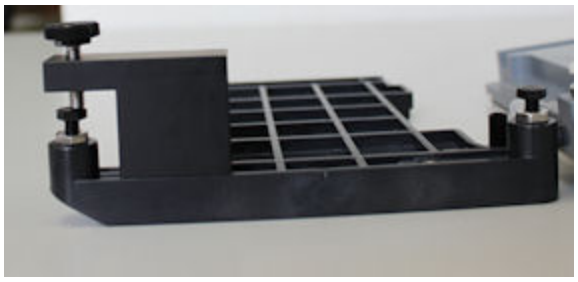
- Measure your factory-installed plate carrier and adjust the vacuum filtration carrier to match its dimensions;
- Level the vacuum filtration plate carrier and set the height of the manifold stop screw;
- Adjust the pressure regulator.

Adjust the Vacuum Filtration Carrier

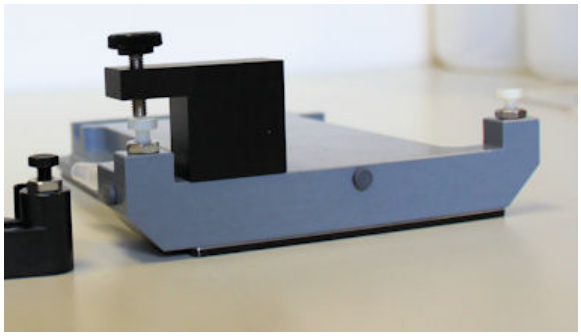
Every plate carrier is customized in the BioTek factory for a specific instrument. The plate carrier's leveling feet are adjusted to precisely fit its EL406. You must imitate this process to customize the new vacuum filtration carrier supplied in the kit to precisely fit your instrument.

Use the **Foot Adjustment Tool** to measure your standard plate carrier's dimensions and adjust the leveling feet of the vacuum carrier to match them:

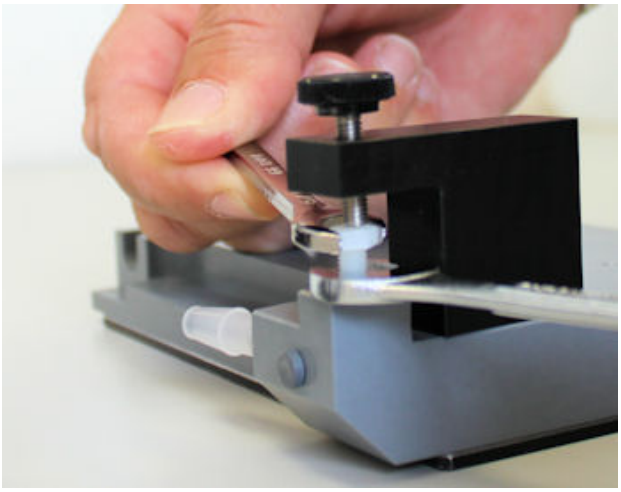
1. Remove the carrier from your instrument and place it upside down on the bench.
2. Unscrew the "manifold stop screw" on the vacuum filtration carrier and place it upside down on the bench in the same orientation as your original carrier.
3. Put the foot adjustment tool on your carrier, aligned with the front nylon foot, to measure its height from the surface of the carrier. Obtain as precise a measurement as possible.



4. Put the foot adjustment tool in the same position next to the front foot on the vacuum filtration carrier.
5. Use the 3/8" wrench to loosen the locking nut of the nylon foot on the vacuum carrier. Turn the nylon foot until its top hits the adjustment tool pin. You should not be able to see light between the foot and the pin.

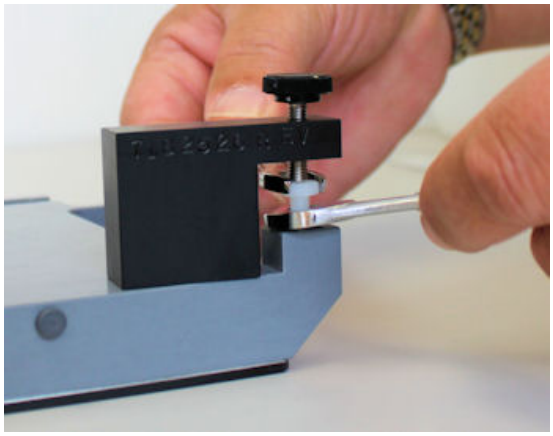


6. Use the 5/16" wrench to hold the nylon foot at the desired height and simultaneously use the 3/8" wrench to tighten the locking nut.



> ▪ Important: The threads on the nylon feet are easily damaged. Do not over tighten the locking nut.

7. Verify the foot is still at the proper height after tightening the nut using the Foot Adjustment tool. Loosen the nut and readjust the foot if necessary, for example, if there is light between adjustment tool pin and the top of the foot.
8. Repeat these steps for the other foot.



Level the Vacuum Filtration Plate Carrier

Make sure the vacuum filtration carrier sits perfectly level on the instrument and does not rock back and forth when one side or the other is pressed down. Readjust the leveling feet to correct any imbalance.

1. Install the vacuum carrier on the instrument and place a microplate with a cover on the carrier. (Remember to seat the carrier in the Y-axis arm.)
2. Use the **Adjust Utility** to lower the wash manifold onto the plate to assess uniformity; to make sure the plate carrier is perfectly level:

LHC	Keypad
1. Select **Tools> Instrument Utilities> Run the Adjust Utility**. 2. Send the Washer to its Aspirate Location.	1. **UTILS > ADJST** 2. Select the plate type. 3. Select **Washer** and **DISP**

LHC	Keypad
The manifold will rest on top of the plate cover. 3. Set the Location Adjustment to Z axis and enter 130 in the **Go to now** field. The manifold will rise a few steps above the plate cover. 4. Click the **Down** button to move the manifold one step at a time onto the plate cover.	position. The manifold will rest on top of the plate cover. 4. Select **MAN** and press ◀ (left arrow) to lower the manifold, one step at a time, onto the plate cover.

3. Closely observe the bottoms of the aspirate tubes looking for differences in the gap between the manifold and the plate. The gap between the surface of a covered microplate and the ends of the aspirate tubes must be uniform across the plate. If not, adjust the front and rear leveling feet.

Important: Do not adjust the foot on the transport rail.

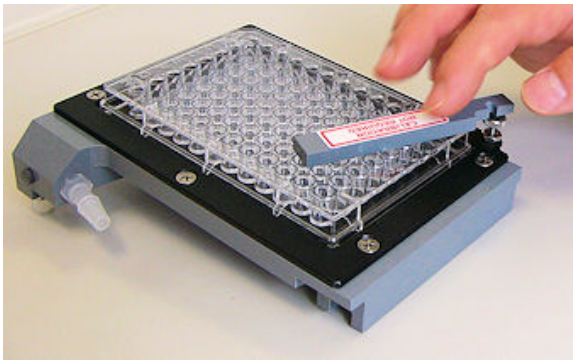
4. If adjustments are needed:
    1. Use the Adjust Utility to lift the manifold so that none of the tubes touch the plate cover.
    2. Use the 3/8" wrench to loosen both locking nuts.
    3. Use the 5/16" wrench to adjust both the front and back feet so that the gap is greater on the left hand side of the plate than on the right hand side.
    4. Beginning with the front foot on the carrier, use the 5/16" wrench to adjust the front foot on the carrier until the left and right sides of the plate are an equal distance from the ends of the tubes.
    5. Use the 5/16" wrench to adjust the rear foot. Adjust the rear foot to remove twist from the carrier and eliminate any rocking of the plate on the carrier.
5. When the gap between the ends of the tubes and the surface of the plate cover is even across the plate and the plate does not rock on the carrier, tighten the locking nuts: Begin with the front foot using the 5/16" wrench to keep the foot from rotating and the 3/8" wrench to secure the locking nut. **Do not over tighten the nut.** Then, use the wrenches to tighten the locking nut on the rear foot.
6. LHC users: use the Adjust Utility to the **Home All** devices. Keypad users: press the Main Menu button to home all the axes.

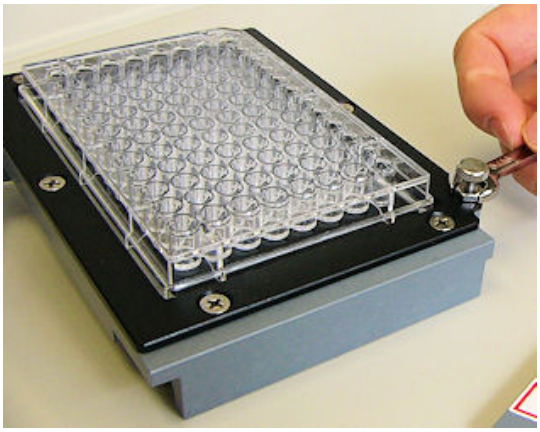
7. With the carrier in the home position, confirm the carrier does not rock on the platform.

Set the height of the manifold stop screw:

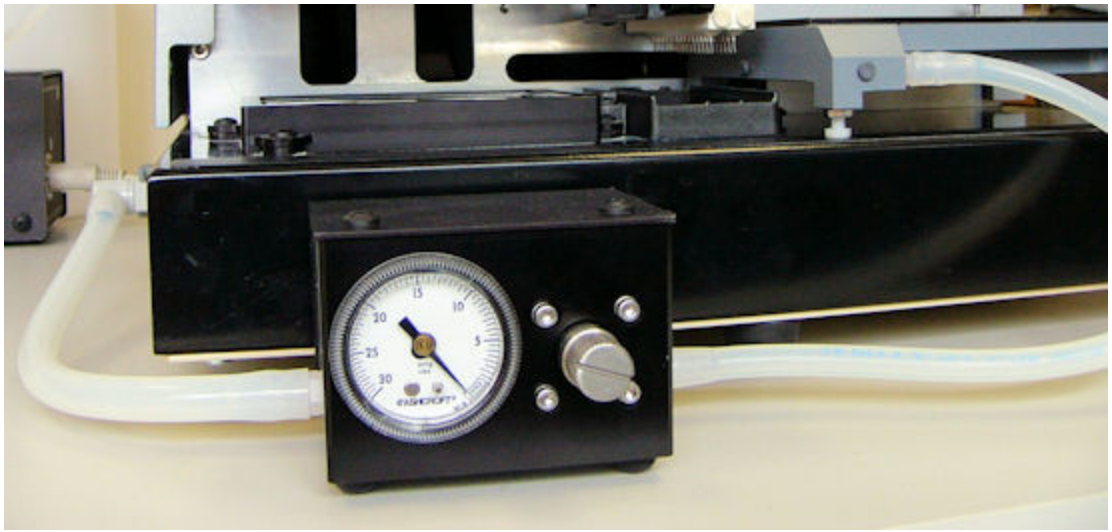
Use the Adjustment jig provided in the kit to set the height of the manifold stop screw.

1. Remove the plate cover from the plate.
2. Place the adjustment jig on top of the plate to ascertain the required height of the manifold stop screw.
3. Turn the thumbscrew to raise or lower it, as needed, until it touches the bottom of the adjustment jig.
4. When the height is correct, use the 5/16" wrench to tighten the lock nut on the carrier.
5. Verify the height with the adjustment jig and readjust if necessary.



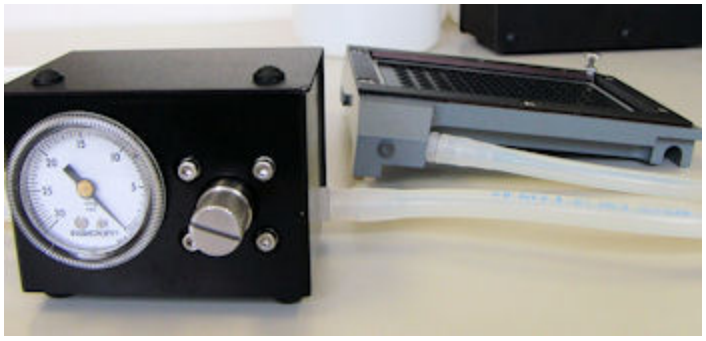


Adjust the Pressure Regulator



Lastly, connect the tubing from the regulator to the plate carrier and from the regulator to the side valve (left side) and perform these final steps to adjust the pressure regulator:

1. Connect the tubing without the quick release valve to the vacuum filtration carrier and to the right side of the regulator. Apply a little alcohol to the ends of the tubing to make the task easier.



2. Connect the tubing with the quick release connector to the left side of the regulator and to the valve on the left side of the instrument.

3. Create an aspiration-only vacuum filtration protocol:

LHC	Keypad
1. Click W-Aspirate **W-Aspirate**. Enable Vacuum Filtration and keep the default time: 30 seconds. 2. Save the file with a unique name, if desired.	1. Select **DEFINE>CREATE** and assign a name, e.g. 30V. 2. Press ENTER to accept the plate type. 3. Select **ADD>WASHR>→>ASPIR** 4. Select **VAC** and keep the default time: 30 seconds.

- LHC users: If the Vacuum Filtration option is grayed out, click **Settings** and "get settings" from the **instrument**. Then, create the protocol.

4. Change the plate carrier setting to run this vacuum filtration protocol:
   - Change the Plate Carrier Setting (Keypad) on page 125 or Change the Plate Carrier Setting (LHC) on page 124
5. Put a standard (non-filter) plate on the vacuum carrier and **RUN** the protocol you just created.

6. While the protocol is running, adjust the regulator, turning clockwise to increase the pressure, counterclockwise to reduce the pressure. Set the regulator to 2.5 inches mercury (inHg), this is the default pressure rating.

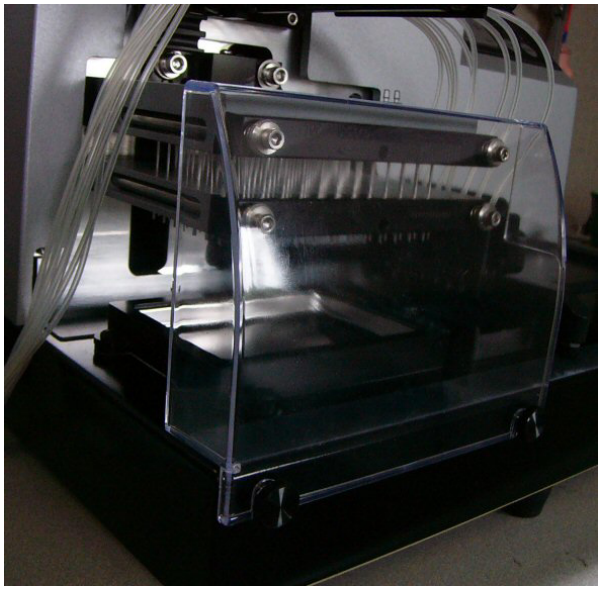
This completes the set up procedure. Now you are ready to run vacuum filtration assays.

Final Check

- Verify that the tubing was not crimped during installation.
- Ensure that there are no loose fittings or cable connections.

Attach the Mist Shield

1. Loosen the two thumbscrews in the front base of the instrument, directly in front of the washer manifold and priming trough.
2. Position the mist shield so the gaps align with the thumbscrews. One side of the mist shield rests on the instrument base.
3. Finger-tighten the two thumbscrews to hold the shield in place.



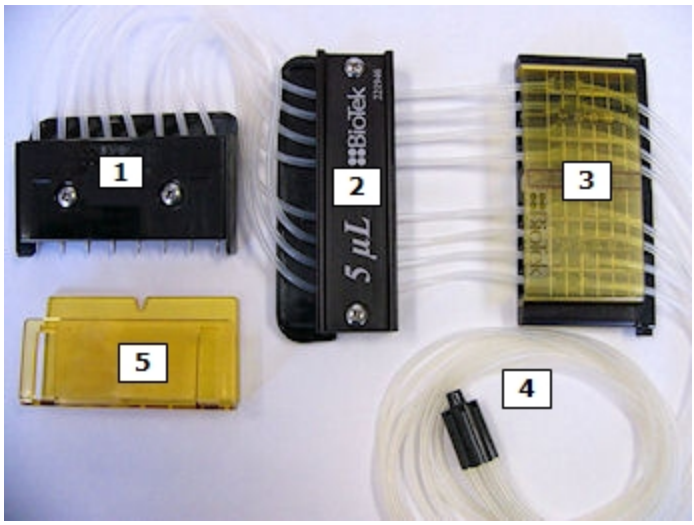
Always lift the mist shield straight up, **not** towards you, when removing it.

Set Up the Peri-pump Dispenser

Install these items to use the Peri-pump dispenser:

- Dispense cassette
- Fluid supply vessel
- (Optional) Prime trough insert

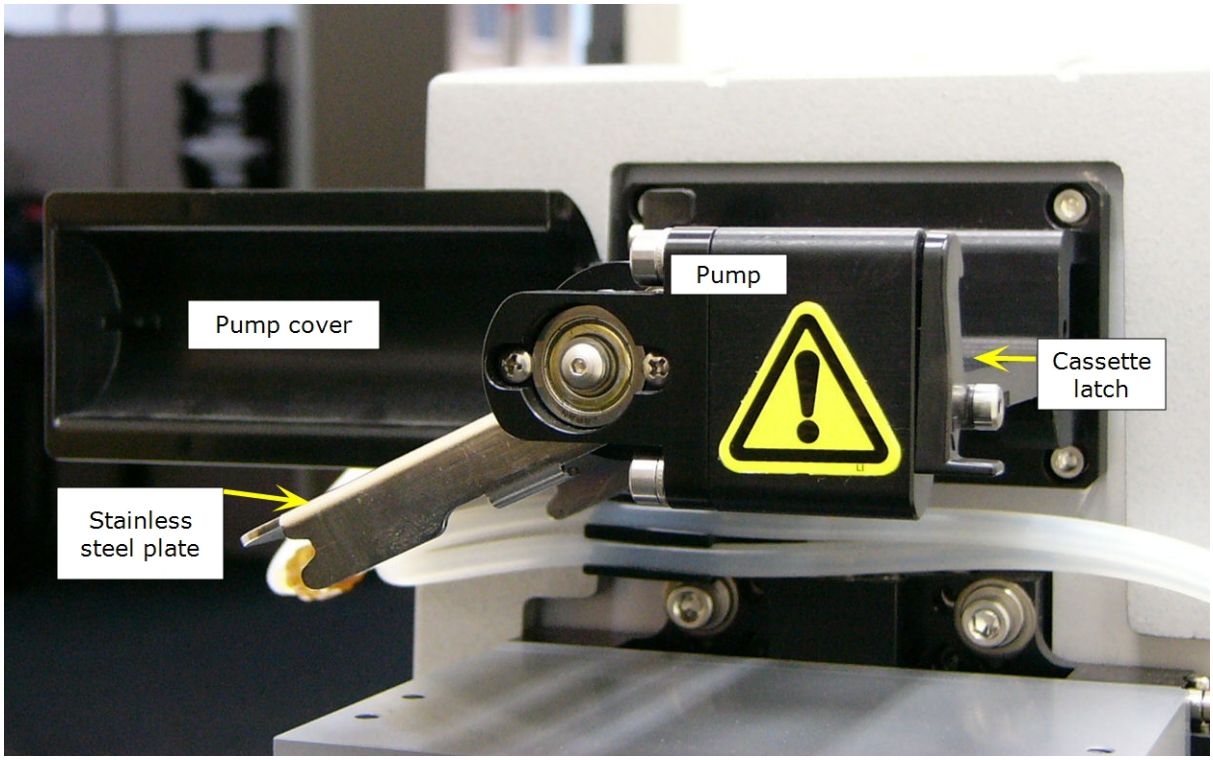
Dispense Cassette Diagram



Tubing Cassette Diagram

1. **Tip Holder**: The cassette's easiest part to identify, the tip holder fits into the dispense arm to the right of the pump for positioning above the plate.
2. **Center Holder**: The center holder is labeled to identify the size of the cassette tubing. It also has a serial number for tracking purposes. It fits in between the tip holder and the tube tensioner and fixes the tubes in place. It slides into grooves on the underside of the pump.
3. **Tube Tensioner:** The transparent 5-mm scale on its front surface identifies the tube tensioner. It has 8 internal screws for stretching the tubing, one for each tube. The tube tensioner's scale is useful when calibrating the cassette.
4. **Tube Organizer**: At the opposite end of the cassette from the tip holder, the tube organizer holds the 8 tubes together for inserting into the fluid vessel.
5. Tip Guard: **Remove** the tip guard before installing the cassette. The tip guard protects the tips during shipping. It is not a permanent part of the cassette.

Install the Dispense Cassette

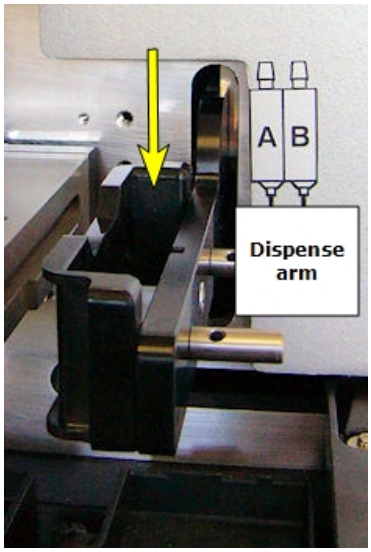


Prerequisites:

- Review the **Dispense Cassette Diagram on previous page** to learn the names of the components.
- Move the **Pump Cover** away from the pump to its **OFF** position.
- Release the pump's stainless steel plate: Use your right hand to release the spring-loaded cassette latch (on the right side of the caution symbol on the pump) and use your left hand to lift the stainless steel plate up and out.

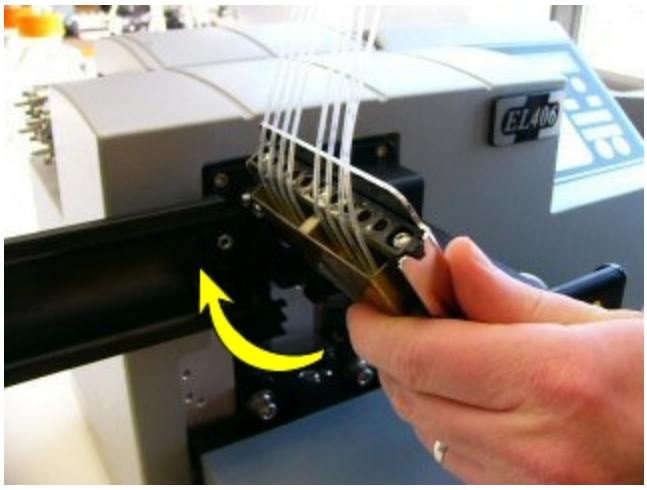
▪ Note: The BioTek logos on the Tip Holder and Center Holder face each other when installed properly. Similarly, the 1µL 1536 cassettes' steel plate on the front of the Tip Holder faces the pump.

1. Slide the **Tip Holder** into the dispense arm. The Tip Holder's front plate with the BioTek logo or steel plate faces the pump. Make sure the tip holder is level and snapped into place.



2. Extend the rest of the cassette under the Peri-pump, so you can slide the **Center Holder** into its slot on the underside of the pump. A tab on the back of the center holder fits into a notch on the pump. The label on the center holder faces down.

3. Align the **Tube Tensioner** with the stainless steel plate as it wraps around and up against the pump. Be sure the knobs on top of the tensioner fit correctly into the grooves in the stainless steel plate as you move both parts up and around the pump and click the steel plate into place.



4. Return the Pump Cover to its **RUN** position covering the pump.
5. Lift the **Tube Organizer** over the pump cover. Place it in the fluid vessel, when you're ready.

- Make sure the instrument's Cassette Type setting matches the installed cassette. **See Change the cassette type setting below**.

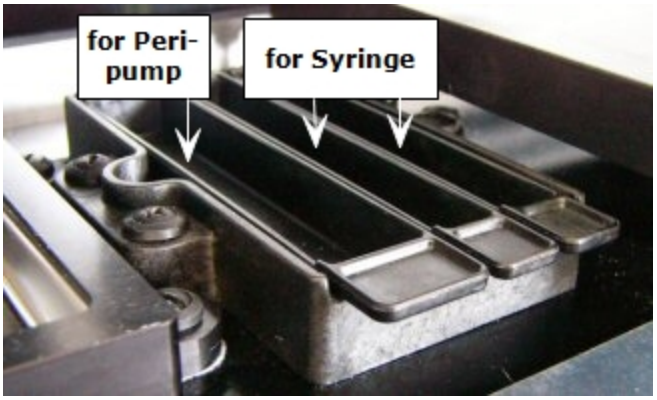
Change the cassette type setting

The Peri-pump **Cassette Type setting** must be correct. The current setting is displayed on the keypad's Main Menu: PER-(#).

LHC	Keypad
1. Select **Tools>Instrument Utilities** 2. Select the **Peri-pump** tab. 3. Select the correct button for the Cassette Type installed. 4. Click **Send** to update the instrument.	1. Press **Setup Menu**. 2. Select **PERI**. 3. Select **PUMP**. 4. Select **CASS**. 5. Select the matching setting. Optionally, use the **Prime** and **Purge** buttons to prepare the cassette for dispensing. 6. Press **Main Menu** to verify the change.

Tip: You may want to employ the Cassette Requirement Mode feature *(as described on page 138)* to automatically update the instrument's Cassette Type setting when you run a protocol. For advanced users with well organized procedures, the EL406 provides the ability to change the cassette type setting on-the-fly. It uses the "Require a specific cassette" parameter in a Peri-pump step to automatically change the cassette type setting if it does not match the required cassette.

Prime Trough Inserts



The EL406 ships with special reservoirs that fit into the dispensers' priming trough to capture expensive reagent after priming, rather than discarding it.

Up to three Prime Trough Inserts are provided:

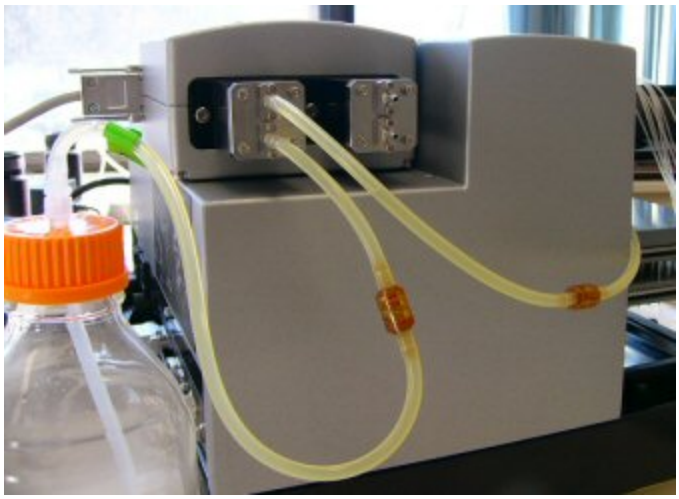
- PN 7182043 for the Peri-pump dispenser holds approximately 12 mL
- PN 7182044 (2) for the Syringe dispensers holds approximately 6.5 mL

▪ Without the prime trough insert, the priming trough empties into the regular waste bottle.

Install the Syringe Dispenser Component

The Syringe dispenser is an optional component and ships separately. Inspect and unpack the shipping container. Skip this part of the installation process if it is not applicable.

Install the Syringe Pumps



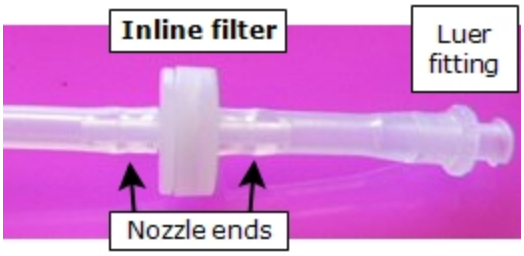
1. Place the unit containing the syringe pumps on top of the EL406 in the hollow designed to hold it (top left, with special indentations for the unit's feet). Alternatively, place the unit on the same surface as the instrument.
2. Plug the interface cable into the back panel of the EL406 in the port labeled **Syringe Pump Dispenser**. Plug the other end into the syringe pump unit.

Install Inline Filter for 32-Tube Dispensers

BioTek ships two 90-micron inline filters with the 32-tube dispense manifolds to reduce the chances of clogging the dispense tubes. It is especially important for the SB – small bore models.

To install the filters:

1. Locate and layout the length of tubing that goes between the supply bottle and the Syringe pump; it has the Luer fitting on one end.
2. Cut the tubing approximately one to two inches (3-5 cm) above the Luer fitting.



3. Slide the filter's nozzle ends into the two ends of the tubing, reconnecting it.

Install Tubing and Manifolds for Syringe Dispenser

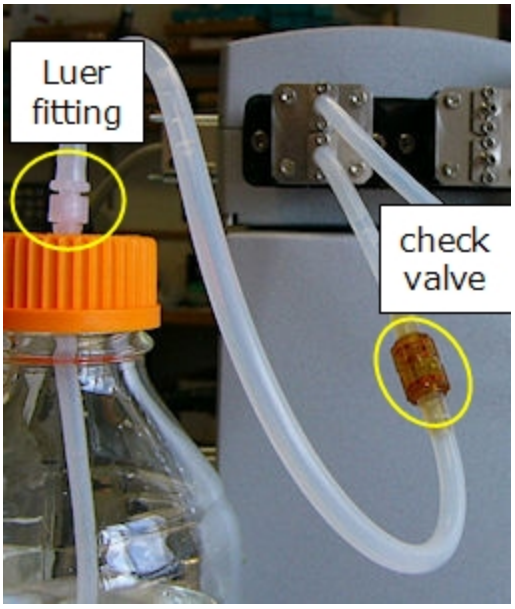
For each dispense pump, a set of two tubes with check valves and two supply bottles are provided. The 32-tube dispense manifolds also ship with an optional inline filter.

> ▪ The supply bottles have Luer fittings. Finger-tighten only!

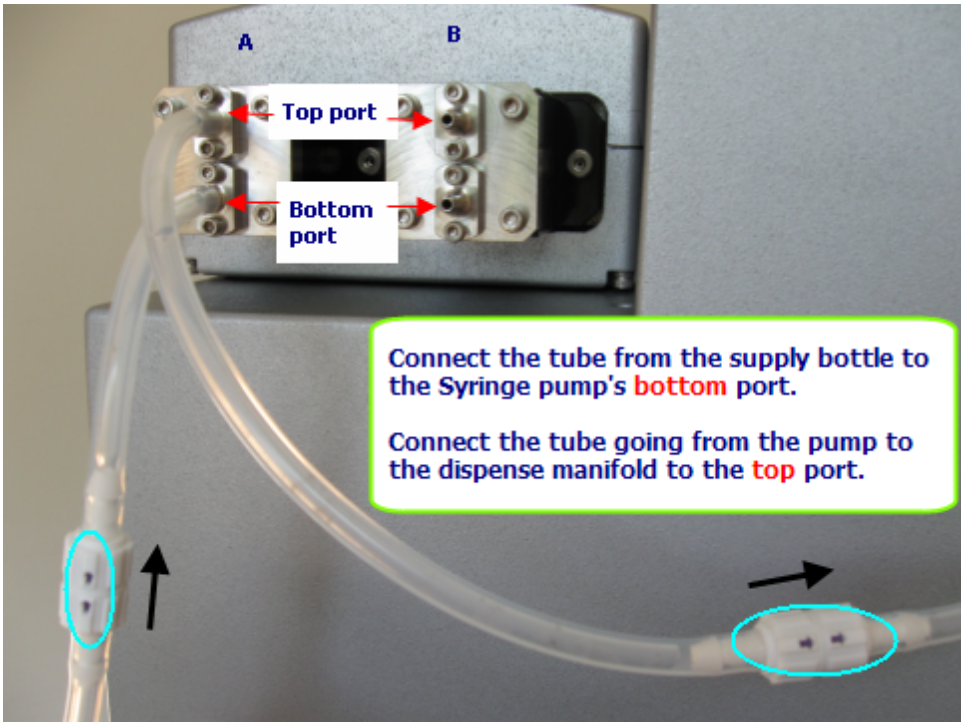
- Rinse all bottles with deionized or distilled water before use to eliminate particles that may have entered during packing or unpacking.
- Place the supply bottles on the same horizontal plane as the instrument. This ensures optimum pump performance.
- Make sure the tubing is not crimped during installation.
- Review instructions for installing the Buffer Switching module in place of the standard supply bottles on page 47, if you purchased this accessory.

Perform these steps twice, first for Syringe A and then for Syringe B:

1. First install the inline filter for 32-tube dispensers, if applicable, *(as described on page 43)*. Locate the tubing with a Luer fitting on one end. Gently screw the Luer fitting into the top of the supply bottle. Finger-tighten only.
2. Attach the other end of the tubing from the supply bottle to the **bottom port** of one of the Syringe pumps.

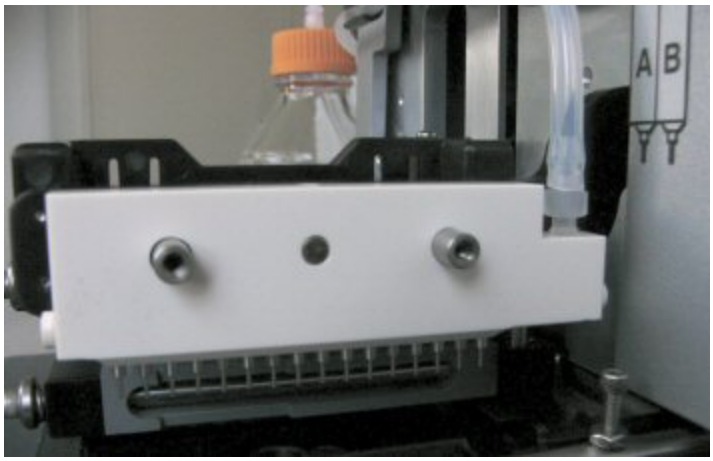


- Make sure the flow-direction arrows point in the direction that the fluid moves.



3. With the check valve's flow-direction arrows pointing away from the pump, connect the other tube (without fittings) to the top port of the Syringe pump.

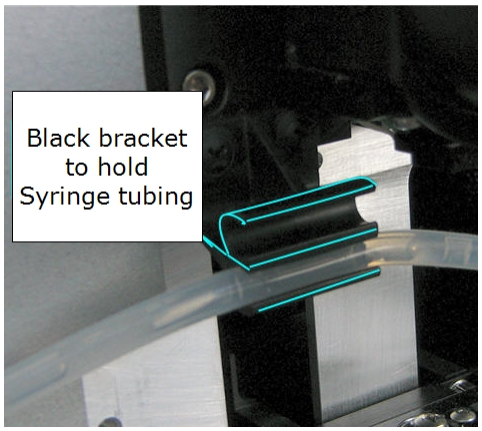
4. Slide the manifold onto the two posts on the dispense arm with the tubing end closest to the instrument. Except for the dual 8-channel manifold that has two manifolds in one block, install Syringe A's manifold first. Syringe B's manifold must be installed after Syringe A's.



5. Connect the supply tube to the dispense manifold.

- Images of syringes on the instrument, labeled A and B, indicate the placement of each dispenser's manifold: A slides on first, then B.

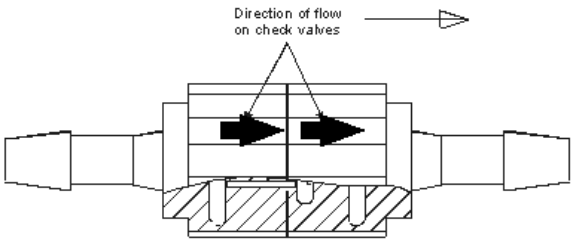
- **Special procedure required for magnetic bead assays!** A magnet holds the two manifolds in place on the dispense arm. You can remove the magnet when necessary for certain assays *(as described on page 145)*.



6. Make sure the manifold has enough slack in the tubing to move down to the priming trough and then press the tubing into the black tubing bracket attached to the instrument just above the wash manifold and below the Peri-pump (if present) to keep the tubing out-of-the-way.
7. Repeat the tubing and manifold installation for Syringe B.

- **Important!** If the dual **Syringe Dispenser** was purchased separately from the instrument, e.g., at a later date, you must tell the EL406 that the Syringe dispenser is installed. **See Update the Instrument to use the Syringe Dispenser on page 52**.

Syringe Dispenser Check Valves



Note the flow direction arrows on the check valves. Some are harder to see than others. The valves are made of a translucent plastic, in which the flow direction arrows are engraved.

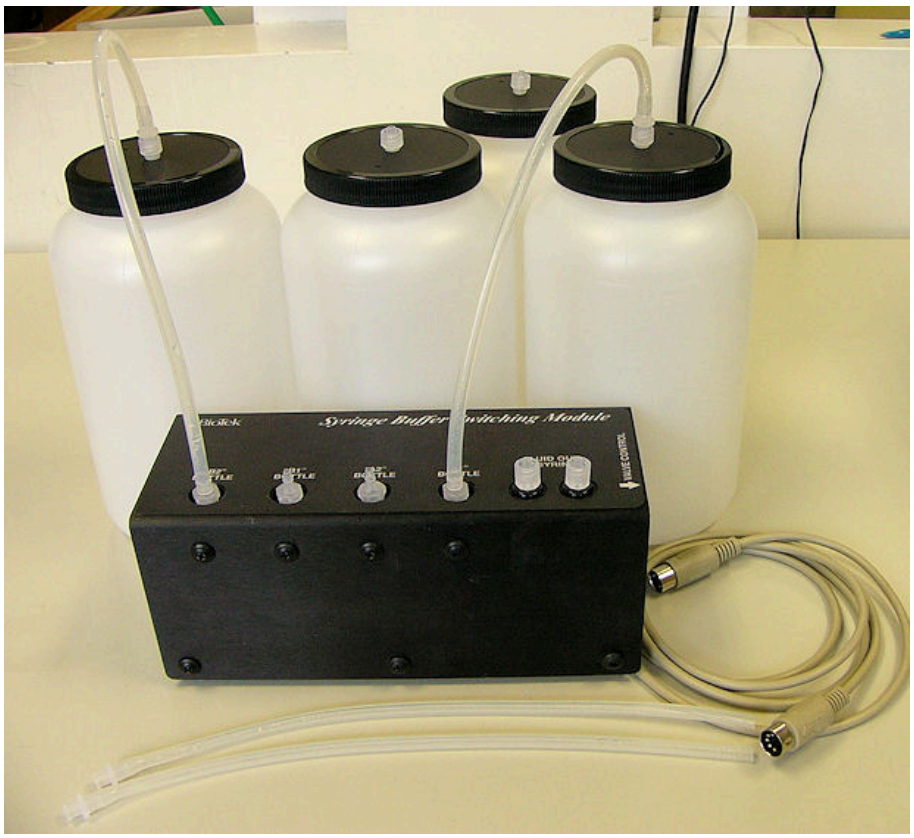
- PN 68083 — Autoclavable valves for use with non-organic substances. The direction arrows are difficult to see.
- PN 68073 — Check valves recommended for use with organic substances. They cannot be autoclaved. Direction arrows are easy to see.

> Note: If the check valves are replaced, it may be necessary to recalibrate the syringe backlash to achieve optimum accuracy performance. **See Calibrate the Backlash for Syringe Dispenser on page 212** for instructions.

Make sure the flow-direction arrows on the check valves point toward the pump from the supply vessel, and towards the dispense manifold from the pump.

Syringe Dispenser Buffer Switching Module

An optional accessory for the Syringe dispenser, this buffer switching module doubles the fluid supply capacity. It lets you set up two supply vessels for each syringe pump.

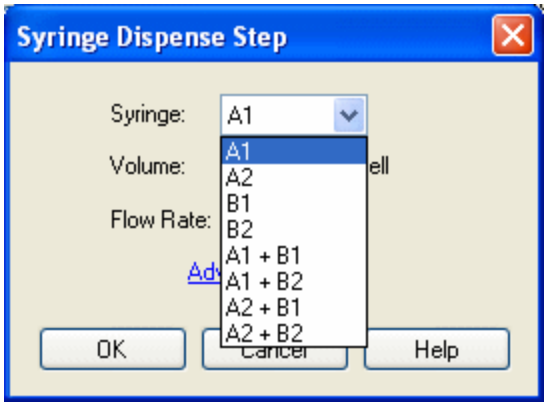


The kit includes:

- Syringe Buffer Switching Module valve box
- 4 supply bottles and 4 tubes with Luer fittings
- Connection cable

About Buffer Switching

Two distinct Buffer Switching kits are available for the EL406. A washer buffer switching module is supplied with certain instrument models ("B" models). In addition, a module for the Syringe dispensers can be purchased as an accessory to the EL406. However, only one Buffer Switching module can be used at a time.



When installed, you will define the fluid source in a protocol to match the valve label of the supply bottle, A1 or A2 for Syringe A, and B1 or B2 for Syringe B, or both. The Quick Dispense options are limited, SYR-A uses valve A1 and SYR-B uses valve B1.

After installing the module, change the instrument settings to enable the feature.

Install the Buffer Switching Module

- Both the washer's Buffer Switching module and the Syringe dispenser's module use the same connection port.

- First install the inline filter for 32-tube dispensers, if applicable, *(as described on page 43)*.

1. Place the four supply bottles and valve box on the same surface as the instrument to optimize performance.
2. Connect the tubing from one of the supply bottles to “A1” Bottle in the valve module.
3. Repeat step 3 with the other three supply bottles for “A2,” “B1,” and “B2” Buffers.
4. Connect Syringe dispenser A's supply tubing (with a check valve and Luer fitting on one end) to the **Fluid Out to Syringe** port A on the valve module. Gently screw the Luer fitting into the valve. Finger-tighten only.
5. Attach the other end of the tubing to the **bottom port** of the Syringe A pump.

6. Repeat steps for Syringe B.
7. Connect the valve cable from the Valve Control port on the module to the Valve Control port on the back of the instrument.
8. Change the instrument's buffer switching setting.

The approximate dead volume for each Syringe dispenser system is 12 mL without Buffer Switching. The Buffer Switching unit adds 5 mL per fluid path. Generally, three times the dead volume completely primes the system. BioTek recommends modifying the predefined prime protocols when using Buffer Switching to account for the difference in fluid paths, e.g. increase the number of prime cycles. When using precious fluids, e.g., expensive reagents, you can change the prime parameters: reduce the volume or number of cycles specified in the predefined protocols or create your own protocols. Use the priming trough inserts to capture expensive reagents when priming.

Change the instrument's Buffer Switching setting

After installing the Syringe Buffer Switching kit (and whenever it is uninstalled), change the setting to tell the instrument its current state:

LHC	Keypad
1. Select **Tools>Instrument Utilities>Syringe Dispenser** 2. Select the applicable button, Installed or not, for the Buffer Switching Module. 3. Click **Send** to update the instrument.	1. Press **Setup Menu** (button in center of keypad). 2. Select **SYR** and then **BUFMOD**. 3. Select Yes when the buffer switching module is installed, otherwise, select No.

- **Important**: Only one buffer switching unit can be installed at a time, either the washer's buffer switching module or the Syringe's. Thus, the instrument's settings for these devices work like a toggle, when one is enabled, the other is disabled, if applicable.

Install Software/Connect to Computer

If you purchased BioTek's Liquid Handling Control™ (LHC) Software to control the EL406 using your personal computer (PC), please refer to the LHC Installation Guide for complete installation and setup instructions.

Connect to Host Computer

Two cables are shipped with the EL406:

If using the serial cable: Plug one end into the **RS232** serial port on the instrument and the other end into an available port on the computer.

If using the USB cable: Plug one end into the **USB** port on the instrument and the other end into an available port on the computer.

- If the computer is connected to the Internet, turn on the instrument. Let Windows® automatically locate and install the necessary USB drivers (follow the online instructions), if applicable or open the link below to download the drivers.
- Virtual Com Port (VCP) drivers for all Windows operating systems are available at http://www.ftdichip.com/Drivers/VCP.htm
- If the computer is NOT connected to the Internet, install the drivers using the supplied "Virtual USB Com Port" driver software CD.

- The keypad must be displaying its "Main Menu" for the LHC to communicate with the instrument.

Technical Note: Only one of the two communication ports (COM port) on the instrument can be used at a time. They cannot be used simultaneously. You can use USB to connect the EL406 to the computer or the RS232 serial port to connect to a BioStack or similar robotic device. But you cannot use both ports simultaneously, i.e. make sure only one cable is plugged in at a time.

Connect to Power

Warning! Power Rating. The EL406 must be connected to a power receptacle that provides voltage and current within the specified rating for the system. Use of an incompatible power receptacle may produce electrical shock and fire hazards.

Warning! Electrical Grounding. Never use a two-prong plug adapter to connect primary power to the EL406. Use of a two-prong adapter disconnects the utility ground, creating a severe shock hazard. Always connect the system power cord directly to a three-prong receptacle with a functional ground.

The EL406 supports voltage in the range of 100-240 V~ at 50-60 Hz.

1. Plug the power cable into the power cable socket in the rear panel of the EL406.
2. Insert the three-prong plug into an appropriate receptacle.

Define Instrument Settings

LHC Users Only

 When using the LHC to control the EL406, an important first step is defining your instrument's settings. After installing the LHC, you can use the desktop icon or the Windows Start button to launch the LHC:

> All Programs> BioTek> Liquid Handling Control

1. Click the **Name** link on the main page and, if required, select the EL406.
2. Specify the COM **Port** used to connect the EL406 to the computer (use the drop-down list to select the port) and click **Test Communication**.
   - **Pass**: proceed to the next step.
   - **Fail**: check the Com Port setting. See "About Com Ports" in the LHC Help.
3. In the Target Instrument Settings dialog that opens, click Get actual settings now, and click **OK**.

Standard Vacuum Pump Users

- **Do not** perform this step when using the **High Flow** vacuum pump (PN 7100754). High flow pumps are recommended when aspirating 384-and 1536-well plates with non-surfactant wash buffers (e.g., pure deionized or distilled water).

Perform this step ONLY if you are using the "standard" vacuum pump (PN 7103024): increase the **Vacuum Dissipate Delay** to match your waste container: 1 second per liter. For example, if you have a 10 L waste bottle, set the delay to 10 seconds.

Using the LHC	Using the Keypad
1. Select **Tools > Instrument Utilities**. 2. Under "General Settings," increase the **Vacuum Dissipate Delay** to match your waste container: 1	1. Press **Setup Menu**. 2. → Select the arrow (for more options) twice.

Using the LHC	Using the Keypad
second per liter. 3. Click **Send** to download this new setting to the instrument. 4. Click **Exit** to return to the main screen.	3. Select **VACDIS**. 4. Use the number pad to set the **Vacuum Dissipate Delay** to match your waste container: 1 second per liter. 5. Press **Main Menu** upon completion.

Update the Instrument to use the Syringe Dispenser

If the dual Syringe Dispenser component was purchased separately from the instrument, you must update the EL406's internal basecode. Conversely, when the Syringe dispenser is ordered with the instrument, BioTek updates the basecode at the factory. Find the calibration data to enter on a label on the bottom of the Syringe pump unit.

LHC	Keypad
1. Connect the syringe pump unit to the instrument with its serial cable. 2. Turn on the instrument, launch the LHC and make sure it is communicating with the EL406 (define the correct COM port). 3. Select **Tools>Instrument Utilities>Syringe Dispenser**. 4. Under Syringe Dispenser Assembly, specify the type of pump and the installed manifold type and click **Send** to download the data to the instrument. 5. In the Calibration Data section, enter the data points on the label in the corresponding fields, including the Serial	1. Connect the syringe pump unit to the instrument with its serial cable. 2. Turn on the instrument, and press **Setup Menu**. 3. Select **SYR > MAN** and specify the installed manifold type. 4. Select **SYR > CAL**. 5. Press ENTER to skip over the Checksum. (You will check this code at the end of the procedure.) 6. Enter the Serial Number (SN) and all the data points by pressing ENTER after each value. Make sure the **CAL VOL** number onboard the instrument matches the **Cal Pt** number on the label. 7. At the Syringe Setup Menu, select **TYPE** and identify the type of

LHC	Keypad
Number and click **Send**. 6. Wait for the confirmation message and verify the Checksum displayed matches the Checksum on the label. This ensures the data was input correctly. • Click **Retrieve** at any time to check the Checksum.	syringe pump, Autoclavable or Non-autoclavable. 8. When finished, select **CAL** again at the Syringe setup menu and confirm that the Checksum displayed onboard matches the Checksum on the label. This ensures the data was input correctly.

▪ **Important**: If the Checksum does not match there was a data input error and dispense accuracy will be compromised. Redo the procedure, carefully comparing the data points on the label to the values entered.

Define Startup Preferences (LHC users only)

You can save enormous time creating protocols by following these steps to define a **New Protocol** template and use it at startup.

Create a protocol template

1.  Click the **New** button or select **File>New**.
2. Click **Name**. Select the EL406 and define its **Port** and **Settings**.
3. Optionally, select the Plate Type, fill in the text fields, and add any steps that you want all new protocols to include.
4.  Click **Save** and assign a unique name, e.g. Template.LHC.
5. Select **Tools>Preferences>New Protocol**.
6. Select the button for Protocol selected below to use as a template.
7. Click **selected** and select the protocol you created as a template.

Define startup behavior:

8. After completing the steps above, select the Startup Options tab.
9. Select the button for **New Protocol**.
10. Click **OK** to save your new preferences.

Verify Performance

Before using the EL406 for the first time, verify that it is operating properly.

- When using the LHC, make sure the EL406 is connected to the PC and both are powered up.
- When running standalone, turn on the EL406.

Using the keypad:

1. Select **UTILS** at the main menu.
2. Select **TESTS** > **SLFCHK**.

Using the LHC:

1. Click the **Name** link on the main page and, if required, select the EL406.
2. Define the COM **Port** used to connect the EL406 to the computer and **Test Communication**.
3. In the Target Instrument Settings dialog that opens, click Get actual settings now, and click **OK**.
4. Select **Tools>Instrument Utilities**
5. On the General Settings tab, click the Perform **Self-Check** link.

Test results:

- **Pass**: no error message is displayed.
- **Fail**: an error message is displayed. If this happens, note the error code and refer to Troubleshooting on page 272 to determine its cause. If the problem is something you can fix, turn off the instrument, fix the problem, and then turn the instrument back on. Otherwise, contact BioTek's Technical Assistance Center.

The Qualification Chapter in the operator's manual provides Installation and Operational Qualification procedures to perform after the instrument is installed and *before* the instrument is used in a laboratory environment.

> **Note:** An **instrument qualification package** (PN 7180527) for the EL406 is available for purchase from BioTek. The package contains thorough procedures for performing Installation Qualification, Operational Qualification and Performance Qualification (IQ/OQ/PQ) and preventive maintenance (PM). Extensive Checklists and Logbooks are included for recording results.

- **Important!** Before operating the instrument, review Optimize Performance on page 65. The guidelines include necessary steps to perform before running a protocol, and issues to consider when creating or editing protocols.

Repacking the EL406

Prior to sending your instrument to us for repair, log into the Customer Resource Center (www.biotek.com) to submit a Service Request for a Return Material Authorization (RMA). Your serial number is needed to process an RMA.

- Failure to comply with the following instructions will void the instrument's warranty. If you have lost the original packing materials, contact BioTek TAC to order Part Numbers 7183000, and if applicable, 7183005 for the Syringe dispenser module, and 7180031 for the vacuum filtration kit.

- Decontaminate the instrument before returning it: **See Decontamination on page 199**.

Prepare the instrument for shipping bracket installation:

LHC:	Keypad:
1. Select **Tools>Instrument Utilities>Washer** 2. Click the **shipping bracket** link under Service Functions.	1. Press **Setup Menu**. 2. Select **WASH** > **PARK**.

3. Remove the Peri-pump's cassette and the Syringe dispensers' manifolds, if applicable.
4. Remove the plate carrier and put it in the accessories box.
5. Uninstall and repack separately the Syringe pump and its accessories, if applicable.
6. Slide the transport rail into position next to the dispense arm. It will be secured in place by one of the shipping brackets.

View the illustrations provided:

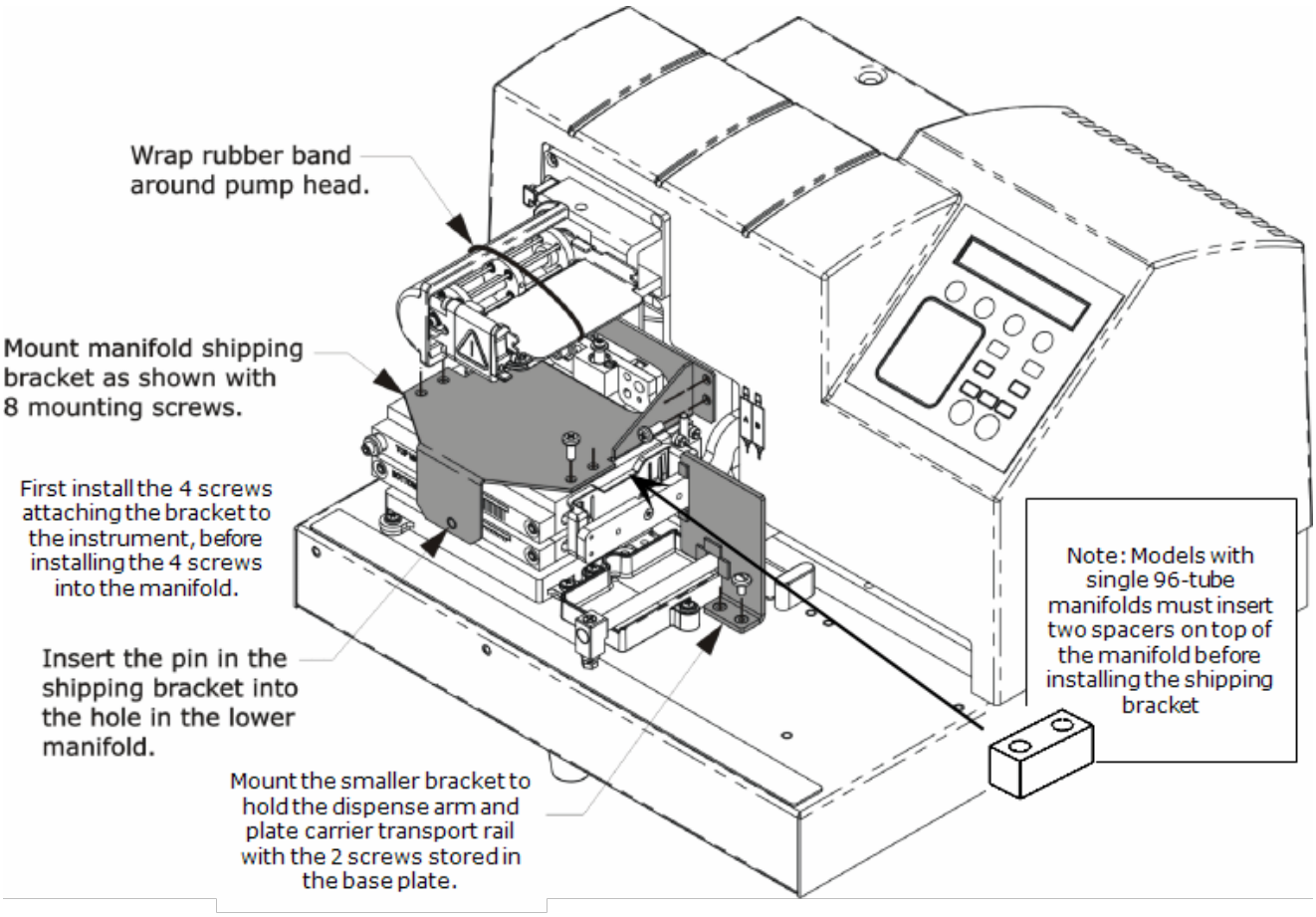
- Reverse the steps described to Remove the Shipping Hardware.
- Repacking illustration 1: install the shipping brackets.
- Repacking illustration 2: put the instrument into shipping boxes.
- Repacking the Syringe Dispenser (if applicable)
- Repacking the vacuum filtration accessories (if applicable)

Obtain an RMA number:

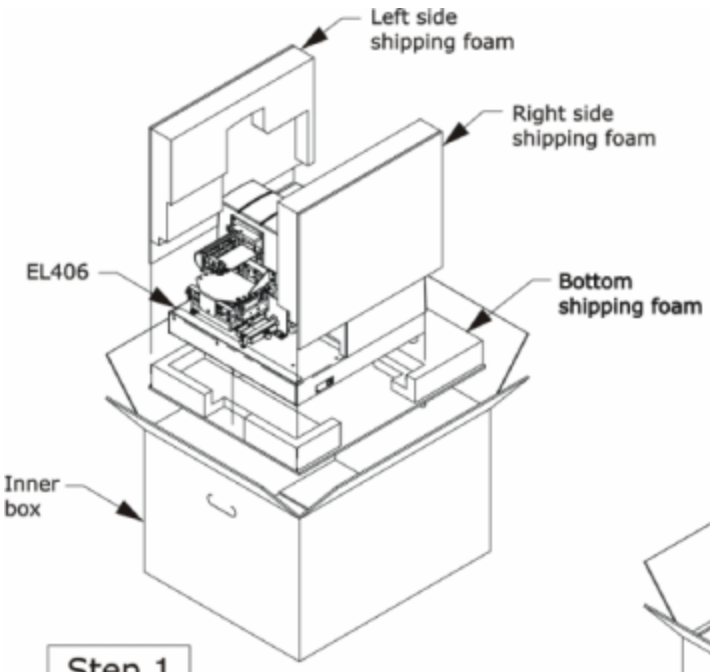
- Contact BioTek TAC to obtain a Return Materials Authorization number,
- Write "RMA" on the shipping box in large, clear letters,
- And, include the RMA number in the shipping address label:

  BioTek Instruments, Inc.
  ATTN: RMA# xxxxx
  15 Tigan Street
  Winooski, Vermont 05404 USA

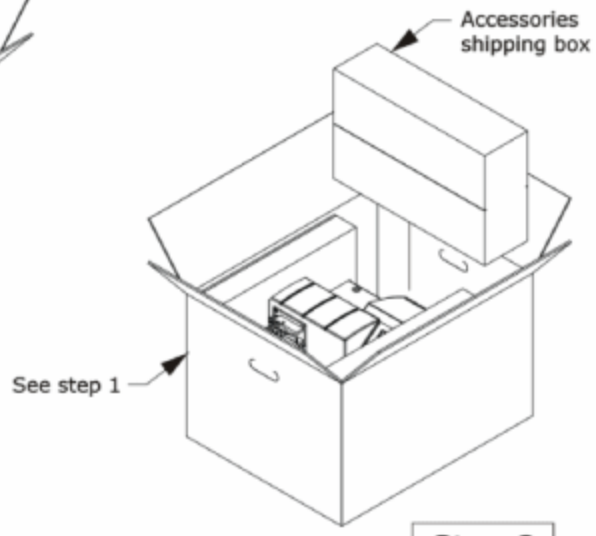
EL406 Repacking



- The instrument's packaging design is subject to change over time. If the instructions in this section do not appear to apply to the packaging materials you are using, please contact BioTek's Technical Assistance Center for guidance.

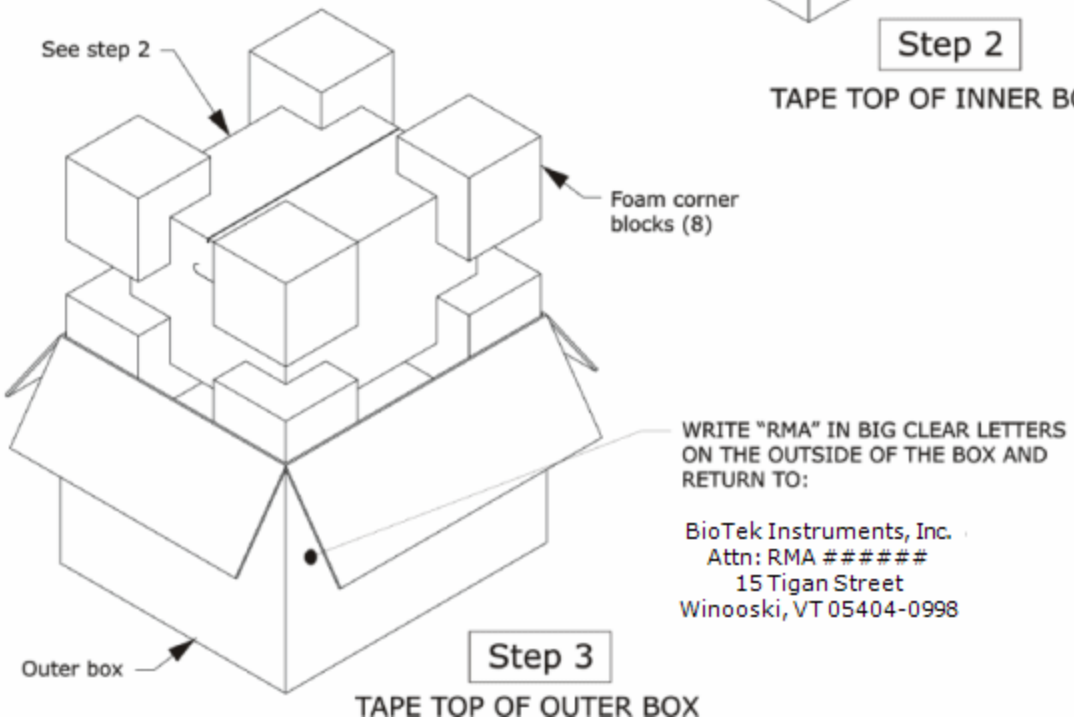


Step 1



Step 2

TAPE TOP OF INNER BOX

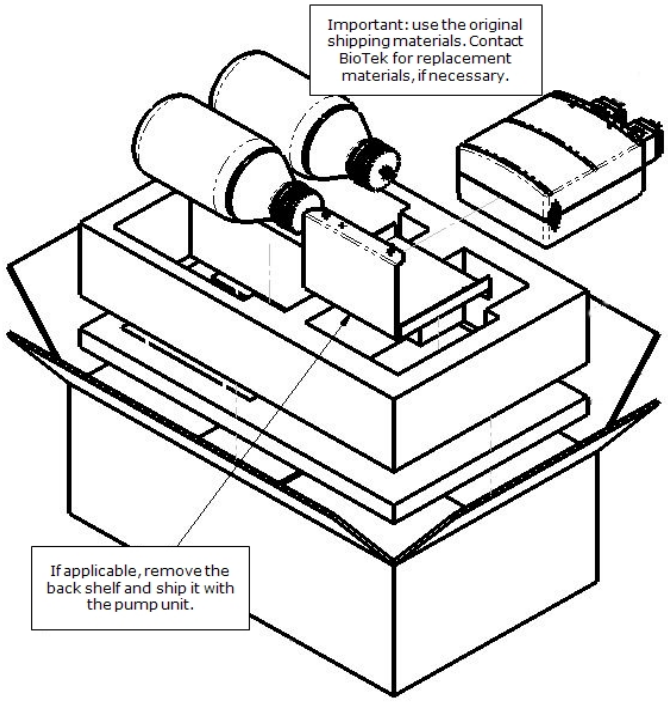


Step 3

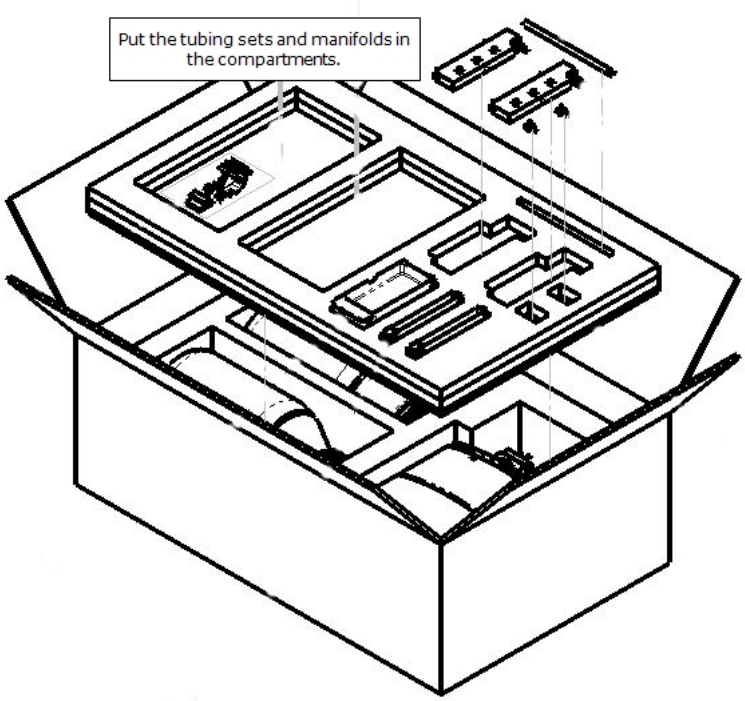
TAPE TOP OF OUTER BOX

Repacking the Syringe Dispenser

After preparing the instrument for shipping, and reversing the installation steps, pack the Syringe dispenser as shown here:

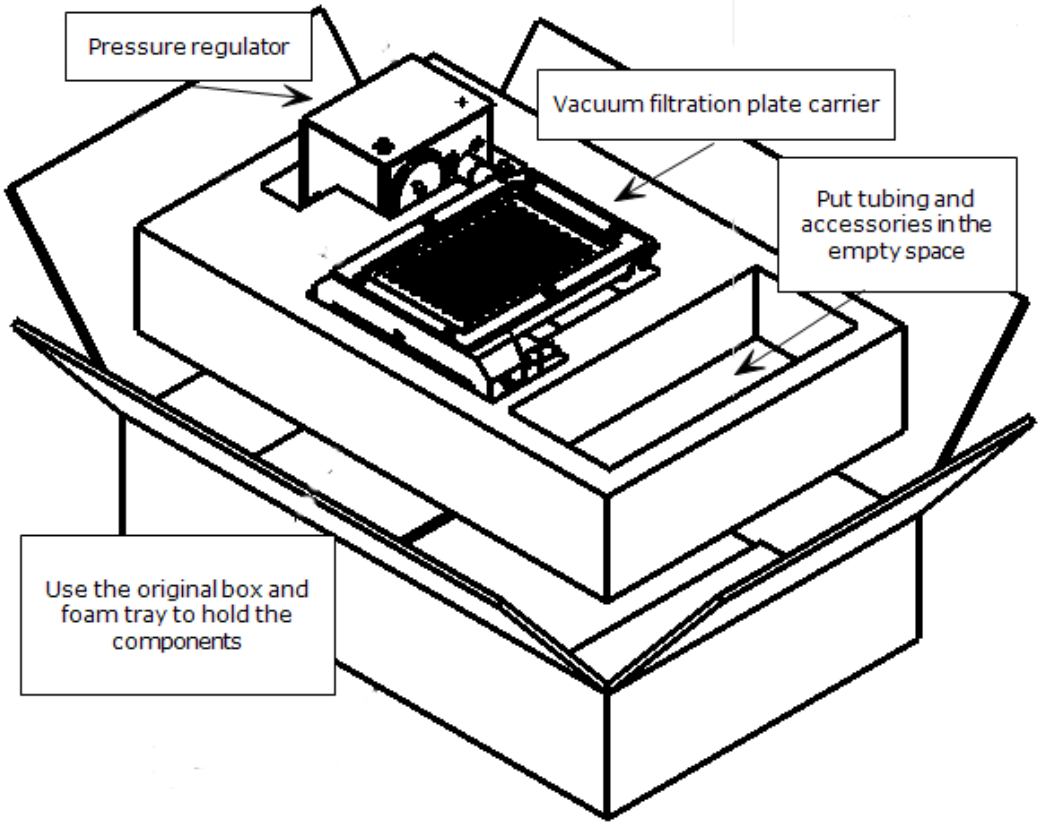


Fill the foam tray with the Syringe dispenser accessories and place the tray on top of the inner foam box containing the pump and supply bottles.

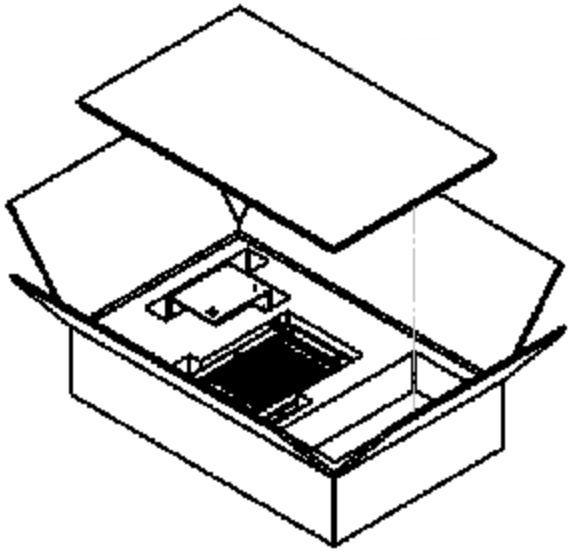


Repacking the Vacuum Filtration component

Reuse the original shipping container to transport the vacuum filtration equipment.



Put the cardboard panel on top of the components and tape the box closed.



Chapter 3

Operation

This chapter provides instructions for controlling the EL406.

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Basic Operation

Two ways to control the EL406

You can control the EL406 using its built-in keypad or with BioTek's Liquid Handling Control™ (LHC) software.

To use the LHC to control the instrument, it must be attached to and communicating with your personal computer (PC), and its main menu must be displayed. Basic protocols can be created or modified using the LHC, and then downloaded to the instrument for stand-alone operation. Learn about transferring protocols from the LHC to the instrument in the LHC Help system: select the **Help** menu or click a **Help** button in a window.

- Find instructions for using the LHC beginning page 96.
- Keypad instructions begin on page 84.

Two ways to wash a plate

The EL406 offers two ways to wash a plate:

- **Quick Wash**: using the keypad you can wash a plate by defining a few parameters like fluid volume and number of wash cycles and rely on default parameters for the more advanced options. **See Quick Wash (Keypad only) on page 86**.
- **Run a Wash Protocol**: using either the keypad or the LHC you can run a wash protocol to wash a plate. You can run a predefined protocol or define your own protocol to specify the optimal parameters for your assay. **See RUN: Running Predefined Protocols on page 67**.

Two ways to dispense fluid to a plate

The EL406 offers two ways to dispense fluid:

- **Quick Dispense**: using the keypad you can dispense to a plate by defining the fluid volume and relying on default parameters for the more advanced options. **See Quick Dispense (Keypad only) on page 88**.
- **Run a Protocol**: using either the keypad or the LHC you can run a dispense protocol to fill a plate. You can run a predefined protocol or define your own protocol to specify the optimal parameters for your assay. **See RUN: Running Predefined Protocols on page 67**.

Optimize Performance

Here are some guidelines to ensure optimal performance and to prevent problems.

Keep the devices clean and the tubing wet

The most critical factor for ensuring optimal performance is to adhere to the Recommended Maintenance Schedule on page 167. Enable **AutoPrime** to keep tubes from clogging.

Prime the tubing to remove air bubbles

- **See Recommendations for Priming the Washer on page 114**
- **See Recommended priming volumes for the Peri-pump on page 135**
- **See Recommended prime volumes for the Syringe dispensers on page 141**

Peri-pump

- **See Release the tension on the dispense cassette on page 136**

Best Practices for all EL406 Devices

- Fill the supply bottles with sufficient fluid.
- **Note:** To avoid spilling fluid when refilling bottles or changing reagents, first release the Quick Connector from the bottle cap, use a paper towel to sop up the few drops in the cap. Then, refill the bottle.
- Make sure the bottles, solutions, and tubing are clean and do not contain any particles or mold. Solutions that are recycled over several days will grow algae, bacteria, molds, or other undesirable organisms.
- Prime before dispensing. Priming the tubing is the most critical factor in assuring optimal performance.
- Empty the waste bottles and firmly seat the bottles' caps and quick release connectors. To make sure fluid does not back up into the vacuum pump during operation keep the waste sensor cable installed and the waste detection sensor activated. If fluid collects in the overflow bottle, thoroughly rinse the fluid-level switch and bottle.
- Check the external tubing connections for kinks and clogs.
- Put microplates on the carrier with well A1 in the left rear corner as you face the instrument, and firmly seat the plate in the carrier.

Before using the Peri-pump

- For top performance and to preserve precious fluids, **Purge** the fluid at the end of a dispense run and **Prime** the tubing before dispensing. The tubing is permeable to air. When 20 minutes or more have elapsed between dispenses, or less than 20 minutes

when using 1 µL cassettes, it is important to thoroughly prime the tubing before dispensing.

- Use the **priming trough insert** to capture expensive reagents for reuse. The Peri-pump's insert can hold up to 12 mL.
- Filter the dispense fluid to 50 microns before dispensing with the 1 µL cassettes. The dispense tips are very small. Filtering the fluid helps prevent clogging.
- **Select the right cassette for the job**: match your desired dispense volume to the recommended cassette. The smallest recommended volume for a cassette type is one aliquot. An aliquot matches the cassette type, 1 µL for the 1 µL cassette, 5 µL for the 5 µL cassette, and 10 µL for 10 µL cassette.
- **Dedicate cassettes for specific fluids** or applications. Reserving specific cassettes for specific uses avoids contamination.
- When the dispenser is idle, **release the Tube Tensioner** element of the cassette from its place on the pump to minimize unnecessary stretching of the tubing. This is especially true for the 1 µL tubing. The best practice is to unload the 1 µL cassette when dispensing is completed.
- To more quickly dispense to 384- and 1536-well plates, use the Instrument Utilities to change the **Dispense Pattern** to Row. If precision is more important than speed, keep the pattern set to Column.

Before using the Syringe Dispenser

- Sufficiently prime the Syringe dispenser to ensure precision and accuracy: **increase the number of prime cycles** to adequately remove all air bubbles from the tubing.
- Use the **priming trough insert** to capture expensive reagents for reuse. Each Syringe dispenser's insert can hold up to 6 ½ mL.

Also see:

- Recommendations for 1536-well hardware on page 114
- Optimize protocols to improve evacuation below
- Cell Wash on page 117

Optimize protocols to improve evacuation

When a wash protocol leaves too much residual fluid in the wells, optimize the protocol with these recommendations:

- Add a secondary aspiration to a wash cycle on page 115, including Final Aspirate,
- Decrease the aspirate Travel Rate,
- Add a Delay to the Aspirate and/or Final Aspirate step ,
- Lower the aspirate height (Z-axis position).

RUN: Running Predefined Protocols

BioTek provides numerous predefined protocols for maintaining the instrument in top condition and for qualifying its performance. Review the **Predefined Protocols on page 69**.

To run a defined protocol:

LHC	Keypad
1. Select **Open** and locate the EL406 folder. 2. Open the EL406 folder to access the more folders. Important: Be sure to Customize the Predefined Protocols below	1. At the main menu, press **RUN**. 2. Press **Options** to scroll to the desired protocol or use the arrow and number keys to enter its number. 3. Press **ENTER** and follow the prompts.

Creating Protocols: Washing, Aspirating and Dispensing Fluid

In addition to the quick routines available from the keypad's main menu, you can define and run protocols. Protocols offer more parameters, giving you the ability to fine-tune instrument performance, and perform more complex processing.

Keypad Control

Find instructions for creating and modifying protocols using the keypad beginning page 89.

Liquid Handling Control™ (LHC) Software

Launch the LHC software to create or modify protocols, see page 96.

Select Help>Help Topics to learn about the LHC.

LHC Users Only: Customize the Predefined Protocols

BioTek provides predefined protocols for maintenance routines and instrument qualification tests. You can quickly customize the protocols for regular use.

The LHC keeps track of the last-used COM port for an instrument type. For example, when an EL406 runs a protocol, the LHC logs the COM port used and the next time an EL406 is used, the LHC applies the same COM port setting. You can disable this feature by defining your Ports preference: select **Tools>Preferences>Ports**.
To correct the COM port for the current protocol, click the **Port** link and use the drop-down list to select the correct value. The LHC stores the COM port value in the protocol file.

With the EL406 connected to and communicating with the host computer (i.e. make sure the instrument is turned on and not busy):

1. Click the **Open** button, locate the **EL406** folder and click **Open**.
2. Open the **Maintenance** or other folder and select the desired protocol.
3. Port: Change the COM port if necessary: click **Port** and enter the correct value or select from the drop-down list.
4. Settings: Click **Settings**, which opens the Instrument Settings dialog.
5. Under Get settings from: click the **instrument** link.
6. Validate Click **Validate**.

   A "Validation successful" message is displayed unless the protocol cannot be run on your instrument. See LHC Protocols Explained on page 99.
7. Save the protocol.

Predefined Protocols Listing

Maintenance Protocols

Daily Maintenance	Description
W-DAY_ RINSE	Simple one-step protocol to fully flush the system with water or reagent to keep the manifold tubes clog-free. Defined for use with Buffer A; 500 mL total volume.
S-DAY_ RINSE_A&B	Two-step protocol to flush tubing for both syringe pumps (A & B). 40 mL per syringe.
P-#UL_CASS_ RINSE	Where # matches the cassette in use. Simple one-step protocol to flush Peri-pump tubing.
W-OVERNIGHT_ LOOP	Protocol designed to keep the manifold in a wetted condition overnight or for a long downtime period; manifold tubes are submerged in fluid for 4-hour intervals between primes in this virtually endless loop. Defined to use Buffer A.
W-RINSE_ AND_SOAK	Identical to W-DAY_RINSE with one addition, the manifold tubes are submerged and soaked for 5 minutes in the fluid.

Periodic Maintenance	
W-Decontaminate W-DECON (onboard)	Aids implementation of the recommended decontamination routine. When using the LHC, this protocol includes prompts for first running disinfectant from Buffer A and later running water through the system from Buffer B.
S-Decontaminate S-DECON (onboard)	This protocol includes prompts for first running disinfectant and later running water through the system; for Syringe A only. It can be easily modified to suit any major cleaning effort.
W-LONG_ SHUTDOWN	Helps implement the routine recommended for preparing the instrument for storage. This protocol includes prompts for running disinfectant from Buffer A, then water from Buffer B, and lastly, air through the system - remove bottle from Buffer C valve.
S-LONG_ SHUTDOWN	Prepares the instrument for long-term storage. This protocol includes prompts for running disinfectant, then water, and lastly, air through the system. Defined to use Syringe A.
S-DAY_RINSE	Identical to S-DAY_RINSE_A&B except only Syringe A is defined.
W-CLEAN_w-BUFFER	Combines priming and AutoClean steps to clean and rinse the

Periodic Maintenance	
W-CLEAN_no-BUFFR	manifold. For units with the Buffer Switching module it is defined to obtain cleaning fluid from Buffer B, and rinse fluid from Buffer A. Units without Buffer Switching are prompted to change fluids.
W-PRIME_200	Simple prime routine; defined for Buffer valve A only.
W-PRIME_ALL_BUFFRS	Consecutively primes each of the Buffer Switching valves beginning with D. Designed for use in the annual instrument verification test of the Buffer Switching module.

QC (Quality Control) Protocols

Manifold-Specific	
W-96_DISP_TEST	Dispense precision test protocol for 96-tube manifold.
W-96_EVAC_TEST	Evacuation efficiency test protocol for 96-tube manifold.
W-192_DISP_TEST	Dispense precision test protocol for 192-tube manifold.
W-192_EVAC_TEST	Evacuation efficiency test protocol for 192-tube manifold.
W-1536_EVAC_TEST	Evacuation efficiency test protocol for 128-tube manifold.
SA-1536_DISP_TEST	Dispense precision test protocol for 32-tube Syringe A manifold.
SB-1536_DISP_TEST	Dispense precision test protocol for 32-tube Syringe B manifold.
P-1536_DISP_TEST	Dispense precision test protocol for Peri-pump 1 µL cassette and 1536-well plate.
W-96_VAC30_TEST	Vacuum filtration evacuation efficiency test for 96-well filter plates and also recommended for use in maintenance procedures.
W-384_VAC10_TEST	Vacuum filtration evacuation efficiency test for 384-well filter plates and also recommended for use in maintenance procedures.

Predefined Sample Protocols

The "Sample" protocols are provided to facilitate learning. Some samples are model specific.

LHC users: You may need to customize the protocols *(as described on page 67)* to match your instrument's settings.

Serial dilutions	
P-96_ DILUTION	Peri-pump serial dilution protocol dispenses 20 µL to 240 µL in 20 µL increments to each column of the plate. 20 µL to column 1; 40 µL to column 2; 60 µL to column 3; and so on till 240 µL to column 12.
P-384_ DILUTION	Serial dilution protocol dispenses 2 µL to 94 µL in 2 µL increments to each column of the plate. 2 µL to column 1; 4 µL to column 2; and so on till 94 µL to column 24.
S-96_ DILUTION	Syringe dispenser serial dilution protocol dispenses 240 µL to 20 µL in 20 µL increments to each column of the plate. 240 µL to column 1; 220 µL to column 2; 200 µL to column 3; and so on till 20 µL to column 12. Defined for Syringe A.
S-384_ DILUTION	Serial dilution protocol dispenses 98 µL to 6 µL in 4 µL increments to each column of the plate. 98 µL to column 1; 94 µL to column 2; 90 µL to column 3; and so on till 6 µL to column 24. Defined for Syringe A.
Cell Wash	
W&P-96_CELL_ WASH	Cell wash-dispense protocol uses the special low-flow tubing, optimal dispense and aspirate heights, and vacuum delay during the wash step. Following the wash, the Peri-pump dispenses 200 µL to each well.
W-CELLWASH_ 96	Cell wash protocol designed to minimize cell layer disturbance in 96-well plates. Aspirate height increased to 50 steps (6.35 mm above plate carrier) resulting in increased residual, approximately 100 µL.
W-CELLWASH_ 384	Cell wash protocol designed to minimize disturbance to cells in 384-well plates. Aspirate height increased to 50 steps (6.35 mm above plate carrier) will cause increased residual volume but help to preserve the cell monolayer.
Microplate Manufacturers	
W-COSTARFLAT	Standard wash protocols modified to best position the manifold tubes for dispensing and aspirating to Corning Costar flat-bottomed and round-bottomed wells.
W-COSTAR_ ROUND	

W-NUNC_384	Standard wash protocols modified to best position the manifold tubes for dispensing and aspirating to Nunc® flat-bottomed wells, round-bottomed wells, and 384-well plates.
W-NUNC_FLAT	
W-NUNC_ ROUND	

Protocol Parameters Tables

For Wash step parameters see Wash Step Parameters Table on page 77.

Peri-pump Parameters

<table>
<tr><th>Step</th><th>Option</th><th>Description/Values range</th><th>Default values</th></tr>
<tr><td>Prime</td><td></td><td>To remove air from the tubing.</td><td></td></tr>
<tr><td></td><td>Volume:</td><td>1-3000 µL</td><td>300</td></tr>
<tr><td></td><td>Duration:</td><td>1-300 seconds</td><td>3</td></tr>
<tr><td>Dis-pense</td><td></td><td></td><td></td></tr>
<tr><td></td><td>Volume</td><td>The per-well volume to dispense:</td><td>10</td></tr>
<tr><td></td><td></td><td>1 µL cassette = 1 - 50 µL and 0.5 µL (HLF-D) for some models*</td><td></td></tr>
<tr><td></td><td></td><td>5 µL cassette = 5 - 2500 µL</td><td></td></tr>
<tr><td></td><td></td><td>10 µL cassette = 10 - 3000 µL</td><td></td></tr>
<tr><td></td><td>Flow rate:</td><td>The rate, µL/second/tube, that fluid is dispensed for each cassette type:
<table>
<tr><th>Cassette Type</th><th>1 µL</th><th>5 µL</th><th>10 µL</th></tr>
<tr><td></td><td colspan="3">(µL/sec/tube)</td></tr>
<tr><td>Low</td><td>50</td><td>120</td><td>140</td></tr>
<tr><td>Medium</td><td>60</td><td>140</td><td>160</td></tr>
<tr><td>High</td><td>64</td><td>160</td><td>180</td></tr>
</table>
When dispensing 0.5 µL with the 1 µL cassette the average flow rate values are:
<table>
<tr><th>1 µL Cassette</th><th>0.5 µL Dispense</th></tr>
<tr><td></td><td>(µL/sec/tube)</td></tr>
<tr><td>Low</td><td>52</td></tr>
<tr><td>Medium</td><td>54</td></tr>
<tr><td>High</td><td>56</td></tr>
</table></td><td>High</td></tr>
<tr><td></td><td>Cassette type required?:</td><td>To require a specific cassette type for this protocol. If yes, select the type, if no, select ANY. See Peri-pump Settings to learn more.</td><td>ANY</td></tr>
</table>

Step	Option	Description/Values range	Default values
	Plate type:	Select the plate type, and optionally, limit the columns dispensed to. For high density plates, skipping certain "Rows" sections is supported, see Peri-pump Dispense Pattern on page 109.	96
	Posi-tioning:	X- and Y- horizontal axes, Z- height (vertical) axis can be adjusted to improve performance. X- and Y-axes default to 0 steps for all plate types.	Z = plate type depend-ent
	Pre-dispense:	Also called "tip priming," pre-dispense normalizes the tips to ensure precise fluid distribution. It dispenses into the priming trough immediately before filling the plate. Pre-dispense is recommended for most applications. Set volume and number of pre-dispenses, except, when dispensing 0.5 µL, the volume is preset: 0.5 µL for 4 cycles by default.	10 µL 2 cycles
Purge		To preserve fluid in the tubing by pumping it back into the supply vessel, i.e. reverses the flow direction.	
	Volume:	1-3000 µL	300
	Duration:	1-300 seconds	3

Note: *Half-microliter (0.5 µL) dispensing requires a 1 µL cassette. The Cassette Requirement Mode behavior is implemented whenever a 0.5 µL volume is requested. Make sure a 1 µL cassette is installed and the Cassette Type setting matches it. When supported, only a 0.5 µL dispense volume can be requested, not 1.5 or 2.5 µL, for example. Perform two dispense steps, one for the half microliter and another at a full increment to achieve these volumes.

Syringe Dispenser Parameters

Step	Option	Description/Values range	Default values
Prime			
	Flow rate:	1-5 (See dispense step description below)	5
	Volume:	80-8000 µL. To remove air from the tubing.	5000
	Syringe:	A or B	A
	Cycles:	Number of prime cycles to perform	2

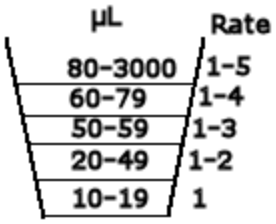
Step	Option	Description/Values range	Default values
	Pump delay:	0-5000 msec.	0
		When dispensing highly viscous fluids, the tubing's check valves perform more slowly. Delaying the syringe pump sufficiently to allow the specified amount of fluid to pass through the check valves before being pumped into the syringe has been shown to improve dispense accuracy. Begin by setting the delay to 500 msec. Experiment with different settings to determine the optimal value for your fluid.	
	Submerge tips:	To soak dispense tubes in the priming fluid for a specified duration for cleaning or maintenance purposes. If yes, set duration, up to 24 hours, in minutes. **See Syringe Prime Step** to learn more.	0
Dispense			
	Flow rate:	Rates 1-5 are dependent on the volume and plate type, except for 1536-well plates. See below.	2
	Dispense Volume:	5-3000 µL depending on the plate type.	10
	Syringe:	A or B or Both	A
		With **Buffer Switching** installed, buffer valves A1 and A2 supply Syringe A, valves B1 and B2 supply Syringe B. When both dispensers are used simultaneously a combination of valves is offered.	A1
	Columns:	Select the plate type, and optionally, limit the columns dispensed to.	96
	Pump delay:	(Same as Prime step description above.)	
	Positioning:	X- and Y- horizontal axes, Dispense height (Z- or vertical) axis can be adjusted to improve performance. Default values plate type dependent; 1 mm higher than the plate height.	
	Pre-dispense:	When enabled, dispenses into the priming trough immediately before filling the plate. Pre-dispense is recommended for most applications. It normalizes the tips to ensure precise fluid distribution. Set volume	10 µL 2 cycles

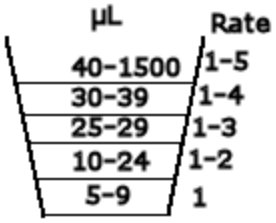
Step	Option	Description/Values range	Default values
		and number of pre-dispenses.	

Syringe Dispenser Flow Rates:

Rates are volume and plate-type dependent:

For example, rate 1 must be used when dispensing between 10-19 µL to a 96-well plate. When dispensing 20-49 µL to a 96-well plate, you can use rates 1 or 2. And, when dispensing 50-59 µL to a 96-well plate, you can use rates 1, 2, or 3. And so on, as shown in these tables.

96-well plate			16-Tube	8-Tube
	**Volume (µL)**	**Rate**	**µL/sec/well**	
	10-19	**1**	450	140
	20-49	1-**2**	600	209
	50-59	1-**3**	750	279
	60-79	1-**4**	900	350
	80-3000	1-**5**	1000	420

384-well plate			
	**Volume (µL)**	**Rate**	**µL/sec/well**
	5 -9	**1**	225
	10-24	1-**2**	300
	25-29	1-**3**	375
	30-39	1-**4**	450
	40-1500	1-**5**	500

- Note: For 16-channel syringes the µL/sec/well rate accounts for 2 tubes/well when addressing 96-well plates and one tube/well for 384-well plates.

1536-well plate			
Volume (µL) 3-3000 The 32-tube manifold flow rates do not have minimum volumes. The µL/sec/well for each type of manifold, Small Bore (SB) and Large Bore (LB), is shown:	**Rate**	**SB**	**LB**
	1	56	125
	2	58	150
	3	60	162
	4	62	174
	5	64	187
The default rate is 3.			

See Plate Types Table on page 103 for default Z-axis values or dispense heights.

Wash Step Parameters Table

See Protocol Parameters Tables on page 73 for Peri-pump and Syringe Dispenser parameters. **See Plate Types Table on page 103** for default Z-axis values or dispense heights.

Minimally, a wash step includes an aspirate step followed by a dispense step. Select and define each option to customize the parameters for your assay.

Keypad name	Option	Description/Values range	Default values
CYCLES	Number of wash cycles:	Each wash cycle first aspirates and then dispenses fluid to and from the plate.	3
ASPIR	Aspirate	Vacuum Filtration: Select standard aspiration (Top) or Vac for filtration.	
	Filtration Time:	When applicable, specify duration of vacuum filtration in seconds. 5-999	30
	Travel Rate:	The rate at which the washer manifold travels down into the wells. The selection range is 1 to 5 for non-cell-based assays, from slowest to fastest. With these rates, the tubes slow their descent as they approach the defined aspirate height (Z Position) to aid complete evacuation of the well.	3
		For delicate, cell-based assays, the range is 1CW (cell wash) to 4CW and 6CW. These rates minimize turbulence in the wells. The tubes descend at a constant rate to the specified height. Rate 6CW creates the least disturbance and performs fastest.	
	Delay:	Amount of time the tubes stay at the aspirate height before lifting out of the wells. Define a delay between 0 - 5000 ms. Increasing the delay may improve evacuation of the wells.	0
	Positioning:	X- and Y- horizontal axes, Z- height (vertical) axis can be adjusted to improve performance. Default Z-axis for	Z = 29 for 96-well plates;

Keypad name	Option	Description/Values range	Default values
		384-well plates is 22 steps, 2 for 384-well PCR plates, 1536-well plates is 40 steps.	X & Y = 0
	Secondary aspirate: (except 128-tube manifold)	Also called Crosswise Aspiration. First the wells are aspirated using the position defined above. The aspirate tubes rise and then descend to the secondary position to aspirate again. This option is not available for 1536-well plates.	No
DISP	Dispense		
	Flow Rate: 96-tube & 192-tube manifolds	The rate at which the fluid is dispensed from the tubes. For cell-based assays, use rate 1 or 2 for gentle washing with the 96-tube manifold only. For normal dispensing, the range is 3-11, 3 is slowest and 11 is fastest.	7
	32-tube dispenser:	For 1536-well plate processing, the Syringe dispenser flow rates 1-5.	3
	Volume:	µL/well dispensed range: 96-tube manifold: 50-3000 192-tube manifold: 25-3000 1536-well (32-tube dispenser): 03-3000	µL/well: 96= 300 384= 100 1536= 10
	Buffer:	Buffer bottle selection. A-D	A
	Positioning:	X- and Y- horizontal axes, Z- height (vertical) axis can be adjusted to improve performance. Default Z-axis for 384-well plates is 120 steps; 83 for 384-well PCR plates.	Z = 121 for 96-well plate
	Vacuum Delay	Suspends the vacuum pump until a certain volume is dispensed. This feature is critical to cell wash operations. It delays normal aspiration until the specified volume has been dispensed to the wells. The range is 10 to 350 µL/well.	10
OPTS	Options		
	PRE	Pre-wash options: Pre-dispense, Bottom wash (if applicable)	

Keypad name	Option	Description/Values range	Default values
	MIDCYC	Mid-cycle options: Shake, Soak, Pre-dispense between cycles,	
	POST	Post-wash option: Final Aspirate	
	FORM	Format for washing 384- and 1536-well plates: Plate or Sector	
PRE	Pre-wash	Actions performed before the wash cycles begin	
	Pre-dispense	Quick, small prime to condition the tips before dispensing.	No
	Flow Rate:	96-tube & 192-tube manifolds: The range is 3-11, 3 is slowest and 11 is fastest.	9
	Volume:	µL/tube dispensed range: 96-tube manifold: 50-3000 192-tube manifold: 25-3000 1536-well (32-tube dispenser): 03-3000	µL/tube: 96 = 50 192 = 25 32 = 10
	Number:	For 1536-well- 32-tube dispenser: the number of pre-dispenses to perform.	2
	Bottom wash	Bottom washing adds an initial wash cycle to the specified number of cycles. Fluid is simultaneously dispensed and aspirated to create cleaning turbulence (at the specified height). The manifold descends to aspirate again and ends with a final dispense to fill the wells. Not available for 1536-well plates.	No
	Rate:	Valid range is 3-11. The cell wash rates, 1 CW and 2 CW, which use low-flow tubing, are available but not recommended. Cell wash options are designed for gentle washing, while bottom wash is designed for vigorous washing.	
	Wash Volume:	25-3000 µL/well dispense	250
	Positioning:	X- and Y- horizontal axes, Z- height (vertical) axis can be adjusted. Repositioning the tubes to harder-to-	

<table>
<tr><th>Keypad name</th><th>Option</th><th>Description/Values range</th><th>Default values</th></tr>
<tr><td></td><td></td><td>reach areas of the wells may the improve results.</td><td></td></tr>
<tr><td>MIDCYC</td><td>Between cycles</td><td></td><td></td></tr>
<tr><td></td><td>Shake</td><td>To mix the contents of the plate.</td><td>No</td></tr>
<tr><td></td><td>Duration</td><td>From one second to one hour.</td><td>5 sec.</td></tr>
<tr><td></td><td>Intensity</td><td><table><tr><td>Intensity</td><td>Hertz</td></tr><tr><td>Slow</td><td>7</td></tr><tr><td>Medium</td><td>13</td></tr><tr><td>Fast</td><td>19</td></tr></table></td><td>Medium</td></tr>
<tr><td></td><td></td><td></td><td></td></tr>
<tr><td></td><td>Soak</td><td>Delays wash for the duration to allow fluids in the plate to steep or incubate.</td><td>No</td></tr>
<tr><td></td><td>Duration</td><td>From one second to one hour.</td><td>30</td></tr>
<tr><td></td><td>Home carrier</td><td>To perform the shake or soak in the home position or not. But, the plate carrier is moved home when the total duration of the shake and/or soak exceeds 1 minute. The vacuum pump is turned off in this scenario. Moving the plate home prevents contaminating it with drops from the manifold.</td><td>No</td></tr>
<tr><td></td><td>Pre-dispense between cycles</td><td>To wet or condition the manifold tubes between cycles, which is only needed after a long soak. Same parameters as regular pre-dispense.</td><td>No</td></tr>
<tr><td>POST</td><td>Post wash</td><td>When all cycles are completed.</td><td></td></tr>
<tr><td></td><td>Final Aspirate</td><td>A final aspiration is performed to completely evacuate the wells. Same parameters as regular aspirate step.</td><td>Yes</td></tr>
<tr><td>FORM</td><td>Wash format</td><td>Manner of processing large-format plates</td><td>Plate</td></tr>
<tr><td></td><td>Sector</td><td>Performs the entire wash step on one sector of the plate before it moves to the next sector.</td><td></td></tr>
</table>

Keypad name	Option	Description/Values range	Default values
	Plate	Performs each cycle to the entire plate before it starts the next cycle.	

Operating with the BioStack

If you purchased BioTek's BioStack Microplate Stacker to operate with the EL406, here is some important information about running it:

LHC Control:

- LHC users: connect both the BioStack and the EL406 to the computer and control them with the LHC.
- The LHC lets you design protocols that integrate BioStack controls with EL406 steps. LHC protocols must contain a BioStack loop.
- In the LHC, select **Help>Tutorials**, click **Sections** in the toolbar for a drop-down menu, select **Controlling the Bio-Stack with LHC**. It only takes a couple minutes to complete this interactive demo. It is a great way to learn about the special BioStack features offered with the LHC.

Keypad Control:

- The Quick Wash and Quick Dispense options do not function with the BioStack, i.e. the BioStack will not deliver a plate. You must create a protocol to process plates using the BioStack.
- You can use the Quick Wash and Quick Dispense **Prime** options. This is recommended especially prior to processing plates, to remove air from the tubing.

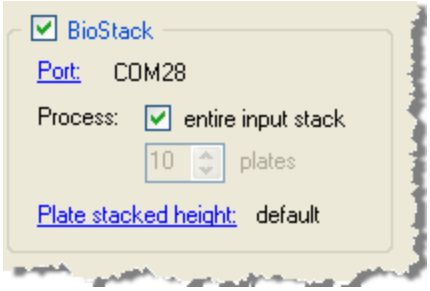
> ▪ Only one of the EL406's communication ports can be used at a time: you can plug in either the USB cable to connect it to the PC or the serial cable to connect it to the BioStack (but not to both at the same time).

Install and Align the BioStack:

1. Set up the BioStack according to instructions in your BioStack Operator's Manual to interact with the EL406. Connect it to the:
   - Host computer (PC) when using the LHC to control the EL406.
   - EL406 when using the keypad to control the instrument.
2. Align the BioStack's gripper with the EL406's plate carrier:

LHC:	Keypad:
1. Select **Tools> BioStack Utilities**. 2. Use the **Alignment Utility**. Click the **Help** button for detailed instructions.	1. Press **Setup Menu**. 2. Select → 3. Select **BIOSTK**. 4. Select **ALIGN**.

3. Set the BioStack operating mode:

LHC:	Keypad:
	1. Press **Setup Menu**. 2. Select → 3. Select **BIOSTK**. 4. Select **CONF**. 5. Select **BIOSTACK**.
Fill the BioStack checkbox in the main view to enable the BioStack action buttons and use them to design a protocol that delivers and retrieves plates.	Important: When using LHC to control the EL406 and the BioStack, the instrument's BioStack configuration setting must be set to **MANUAL**, not "BIOSTACK."

- To Restack or not? Yes: to keep plates in the same order. No: to save time when the plate sequence is unimportant.

5. **Verify** the setup: perform a protocol with 1 or 2 plates.

At the start of the day, power up the BioStack first, and then the EL406. BIOSTACK2WR: Lift the BioStack's gripper before turning it on.

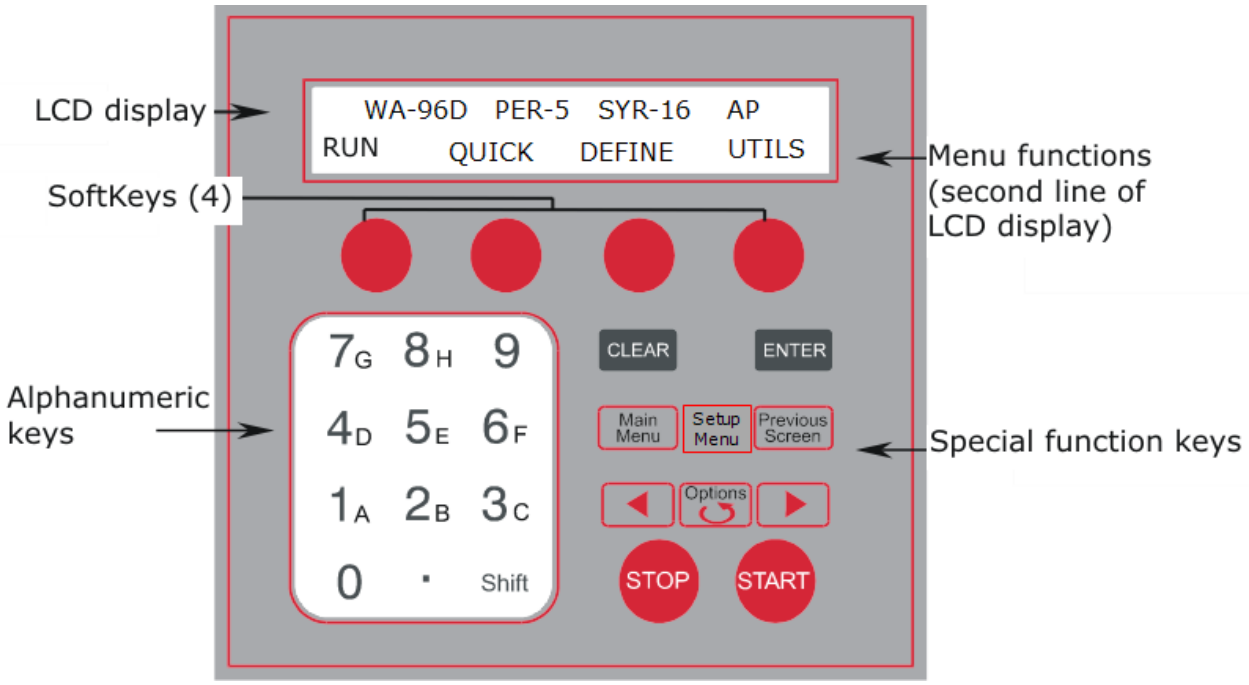
Robotics integrators: CAD drawings of the physical dimensions of the EL406 are available upon request. Contact BioTek customer service.

Technical Note: Only one of the two communication ports (COM port) on the instrument can be used at a time. They cannot be used simultaneously. You can use USB to connect the EL406 to the computer or the RS232 serial port to connect to a BioStack or similar robotic device. But you cannot use both ports simultaneously, i.e. make sure only one cable is plugged in at a time.

Keypad Control: When the BioStack is connected to your EL406, you are controlling both instruments using the keypad. Before connecting the EL406 to your computer to download basecode or for other reasons, you must first disconnect the BioStack from the EL406 and change the Instrument Setting for the BioStack: Press **Setup Menu> →> BIOSTK> CONF>MANUAL**.

Introducing the EL406 Keypad

The keypad on the EL406™ Microplate Washer Dispenser features 26-keys and a 2 x 24-character LCD. The main menu is shown below.



Starting at the top of the keypad, note the main menu and the **Soft-keys**. Use the Soft-keys to make selections. To return to the main menu, press the **Main Menu** key.

At the main menu, the top line displays **AP** when AutoPrime is enabled.

- **RUN** - to run a previously defined protocol. Use the **Options** key to select a protocol or enter its number. **See Predefined Protocols Listing on page 69**.
- **QUICK** – leads to the Quick Wash and Quick Dispense menus. Depending on the devices installed, press **Pump** to scroll to the WASH, PER for Peri-pump, and when the Syringe dispenser is installed, SYR-A, SYR-B, or SYR-BOTH quick dispense menus, if applicable:
  - **Wash** – When WASH is displayed, put a plate on the carrier, set the desired parameters and press **Start** to run a wash routine. **See Quick Wash (Keypad only) on page 86**. Except when the 1536-well hardware is installed and quick wash is not supported.
- **See Quick Dispense (Keypad only) on page 88**:
  - **Peri-pump Dispenser** (PER) options:

- First, make sure the PER-# matches its installed cassette type. If not, change the setting: **See Change the cassette type setting on page 41**.
- Press and hold the Prime and Purge keys to execute these actions for as long as you hold the key.
- Set the dispense VOLume using the arrow and number keys, put a plate on the carrier, and press **Start**.

◦ **Syringe Dispenser**: When the dual Syringe dispensers are installed press **Pump** at the quick menu to toggle through the list of dispensers, Syringe A, Syringe B, or Syringe-BOTH.

- Press the Prime key to prime the tubing with 5000 µL of fluid.
- Set the dispense VOLume using the arrow and number keys, put a plate on the carrier, and press **Start**.

◦ **Turn On/Turn Off the Vacuum Pump** to drain the priming trough. At the Quick menu:

- Press Shift+1 to turn on the vacuum pump.
- Press Shift+2 to turn off the vacuum pump.
- Use these key sequences to manually control the vacuum pump when dispensing fluid. At times the priming trough fills up and is not emptied automatically. This option gives you control in that situation.

◦ **PUMP** – to select the device to perform a quick routine.

◦ **PLATE** – to select the plate type for a quick routine. The default plate type is 96-well. Press PLATE to scroll through and select a plate type. **See Define the Plate Type and Plate Map (or Partial Plate) on page 91** and the Plate Types Table on page 103 to learn about the types of microplates supported.

- **DEFINE** – leads to the protocol creation and editing mode: Create or Edit a Protocol on page 89.
- **UTILS** – to run system tests, the Adjust Utility, and to define AutoPrime parameters.
- **Setup Menu**: press this key to access the instrument's general settings and the settings for each of the devices and the BioStack.
- The **Options** key (and sometimes the arrow keys) scroll through the available options or settings for the current focus. Shift+Options reverses the scrolling direction.

Quick Wash (Keypad only)

1. Select **QUICK>WASH** at the main menu to perform a quick wash (with Buffer A when Buffer Switching is installed).
2. Press Pump, if necessary, to get to the Quick Wash menu.

```
96    WASH:003    VOL:0300 uL
PRIME    PARMS    PUMP   PLATE
```

Quick Wash Menu

- Quick Wash is not an option for 1536-well plates. You must define a protocol or run a predefined protocol.

The EL406 saves the parameters that were last used to run a quick wash. They are displayed in the top-line of the LCD:

- **96** in the above example is the plate type. Select **PLATE** to change it; scroll through the compatible choices for plate washing with the currently installed hardware and press Enter to select the desired plate.
- **WASH:003** is the number of wash cycles (one aspirate and dispense step per cycle). Select **PARMS>CYCLE** to change the number of cycles to perform.
- **VOL:0300 uL** shows the dispense volume per well in microliters.

To change the dispense volume, use the arrow keys to move the cursor to the desired number position. The cursor appears to underline a number: 0010. When the correct position is selected, use the number pad to enter the desired value. Or, press Options to increment the value, Shift+Options to decrease the value.

- **Buffer** is offered on washers with Buffer Switching to select the buffer valve. The current selection is displayed in the top right corner of the LED.
- **PRIME** to flush the tubing to remove air bubbles with the desired volume.
- **PLATE** lets you change the selected plate type and define a partial plate run, if applicable. **See Define the Plate Type and Plate Map (or Partial Plate) on page 91**.
- **PARMS** lets you change the number of wash cycles and the height of the aspiration manifold above the wells.
- **PUMP** changes the selected device; exits the Quick Wash menu.

When the desired values are entered, put a plate on the carrier and press **Start** to run the routine. Up to 10 quick wash routines are saved. When 10 have

been defined, the newest replaces the oldest. Use the **Options** key to scroll the quick wash routines to select one.

If Quick Wash is too limited to satisfy your assay requirements, use the LHC or the keypad to define a wash protocol. All wash parameters can be defined during protocol creation, including those designed for special assays.

Quick Dispense (Keypad only)

1. Select **QUICK** at the main menu to perform a quick dispense.
2. Press **PUMP** (if necessary) to select the dispenser you want to use. Depending on how many dispensers are installed, pressing Pump scrolls to the WASHer, PER for Peri-pump, or when the Syringe dispenser is installed, SYR-A, SYR-B, or SYR-BOTH quick dispense menus, if applicable.

```
96    SYR-A      VOL:0100 uL
PRIME          PUMP   PLATE
```

Quick Dispense Menu

The EL406 displays the last **Quick Dispense** that was run for the selected device:

- **96** (displayed above) is the plate type. Select **PLATE** to change it; scroll through the compatible choices for plate with the currently installed hardware and press Enter to select the desired plate.
- **PER-#** is the Peri-pump cassette setting. Make sure it matches the currently installed cassette: **See Change the cassette type setting on page 41**. Refer to instructions for physically changing the cassette: Install the Dispense Cassette on page 39.

  > ▪ 0.5 µL dispensing is **not** an option via the Quick menu. You must define a protocol (Half microliter)

- **SYR-A** or **SYR-B** or **SYR-BOTH** identifies the Syringe dispenser to be used in the Quick Dispense.
  - When the Syringe Buffer Switching module is installed, SYR-A uses valve A1 and SYR-B uses valve B1.
- **VOL:0010 uL** shows the dispense volume (not the priming volume) per well in microliters (µL).

To change the **dispense volume**, use the arrow keys to move the cursor to the desired number position. The cursor appears to underline a number: 0010. When the correct position is selected, use the number pad to enter the desired value.

- **Prime**: At the Peri-pump dispense screen, press and hold the prime key to prime the tubing. Fluid flows into the prime trough for as long as you press the key. For the Syringe dispensers, **PRIME** pumps 5000 µL each time it is selected.

- **Empty the priming trough:** Turn on/off the vacuum pump when using the Quick Dispense menu: **Shift+1** to turn it on; **Shift+2** to turn it off.
- **Purge**: At the Peri-pump dispense screen, press and hold the Soft-key to purge the tubing. Fluid is pumped back into the supply vessel as long as you press the key.
- **Plate**: lets you to change the plate type and plate map or, more accurately, the columns of the plate to dispense to. **See Define the Plate Type and Plate Map (or Partial Plate) on page 91**.

> ▪ **153F**: For best performance with this plate type use SYR-BOTH (both Syringe A and B) and remove the Peri-pump dispense cassette, or remove the Syringe manifolds when dispensing with the Peri-pump.

When the desired values are entered, put a plate on the carrier and press **Start** to run the routine. Up to 10 quick dispense routines are saved for each dispenser. When 10 have been defined, the newest replaces the oldest. When the desired dispenser is displayed onscreen, press the **Options** key to scroll through the quick routines to select one.

Create or Edit a Protocol (Keypad Only)

At the main menu:

1. Select **DEFINE**, and then, **CREATE** or **EDIT**.

	CREATE	**EDIT**
2.	**Name** the protocol and select the **Plate Type**. Press **Enter** after making selections to proceed. **See How to name a protocol (Keypad only) on next page**	Select the protocol to edit: enter its number or use the **Options** key to scroll through the stored protocols to select one. Then, you can edit the name and plate type, if desired. Press **Enter** to proceed.

3. Define or modify the plate type using the Previous and Next buttons to scroll through the supported Plate Types.

4.	Select **ADD** to define the first step: then, select the device to use or **SHAKE** to mix or soak the plate's contents.	**EDIT** the first step or press the **Options** key to scroll to the step you want to change in a multi-step protocol.

5. Select the device to use:

WASHR	To use the wash manifold to wash, dispense or aspirate fluid or to run AutoClean.

PERIP	To dispense fluid using the Peri-pump dispenser. Select HLF-D for 0.5 µL, if applicable.
SYRNG	To dispense fluid using one or both of the Syringe dispensers, A or B.
SHAKE	To mix the contents of the plate and/or soak or steep the fluids for a specified time period.

5. Select the action you want the device to perform and then define the
6. step's parameters. Press **Enter** to proceed.

6. Keep Added Step? | Save Step Changes?
7.

   Select **Yes** or **No** using the Soft-keys to save or discard your inputs for the current step.

* Press **Main Menu** to end the session at any time. Then, select **RUN** and select the protocol to run it.

See Protocol Parameters Tables on page 73 for valid ranges.

How to name a protocol (Keypad only)

At the **Name** screen when you are creating or editing a protocol, you can enter up to 16 alphanumeric characters to name the protocol:

- Press **Shift** + the number key for **A-H**, or scroll through the alphabet with the **Options** key for **A-Z**.
- Press **Shift +Options** to reverse direction.
- Use the arrow keys ◄ ► on either side of the **Options** key to move the cursor within the display.
- Press its Soft-key to add one of the four symbols (- % & _) in the display to the protocol name.
- Press **ENTER** when you are finished to store the protocol name.

- If the name already exists, an Invalid Protocol Name message displays and you must enter a unique name.

Define the Plate Type and Plate Map (or Partial Plate)

The EL406, depending on the currently installed hardware, can process 96-well, 384-well, and 1536-well plate types. Washing and dispensing to a part of the plate is limited by the foot print of the hardware. When washing 384- and 1536-well plates, you can choose the sectors to be processed or skipped. When dispensing you can skip certain columns in any plate type. This requires defining a "plate map." By default, the whole plate is processed.

Quick Routines: Quick Wash and Quick Dispense

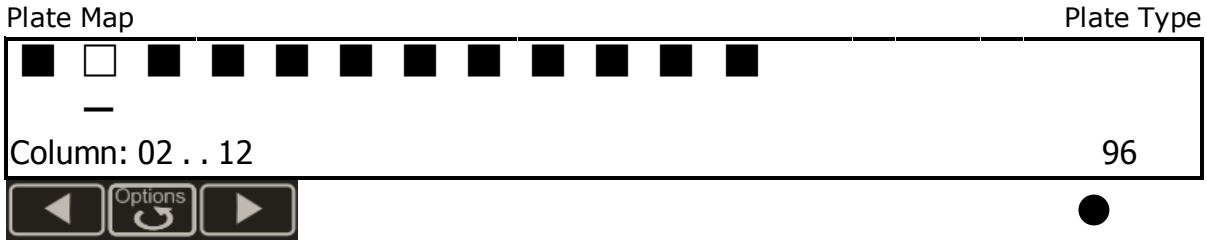
- When defining a quick routine, select **Plate** with the Soft-key to set the plate type, and to define the plate map.

Protocols: Wash and Dispense

- When creating or editing a protocol (Define mode), set the **Plate Type** after naming the protocol. Use the **Next** or **Previous** button to scroll through the options.
- To define the plate map (i.e. the columns, and rows when using the Peri-pump):
  - **Wash** step: select **OPTS** > **FORM**. First choose the processing mode Sector or Plate (described below). Then, proceed to the plate map screen.
  - **Dispense** step: press Enter to proceed to the plate map screen.

To change the plate type:

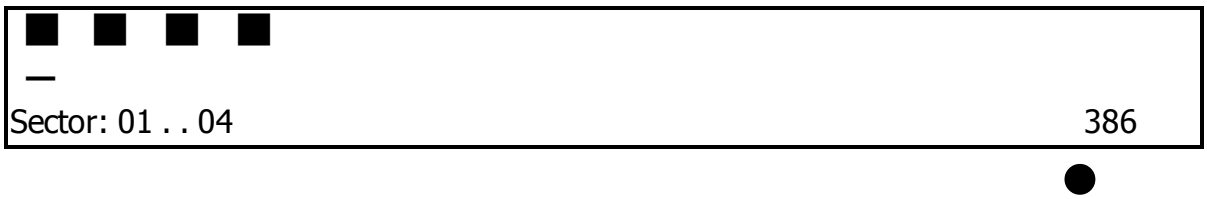
- **Quick Menu**: Repeatedly press the Soft-key under the plate type (in the right corner of the display) to scroll through the available options. Hold the Shift key while pressing the button to reverse direction.



The two-line display changes to show a representation of the current plate type's columns or sectors in the top line. The display shows each column as a filled or empty square; empty columns/sectors will not be dispensed to or washed. For the current plate type, shown in the right corner, the left corner shows the

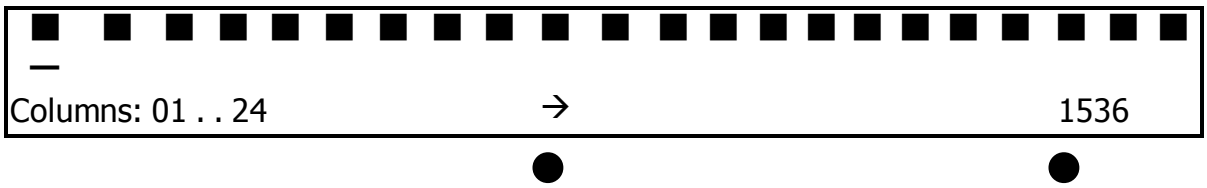
selection range, beginning with the currently selected column/sector. (In the example above, column 2 is currently selected to be skipped.)

- **DEFINE mode**: When defining a protocol, press **Next** or **Previous** to specify the plate type.



The two-line display changes to show a representation of the current plate type's sectors in the top line, with each sector as a filled or empty square; empty sectors will not be washed. Press the **Options** button to toggle the sector to filled or empty.

Press the **Clear** button once to empty all the columns. Press it again to fill all the columns. This is useful when you want to dispense to only a couple columns.

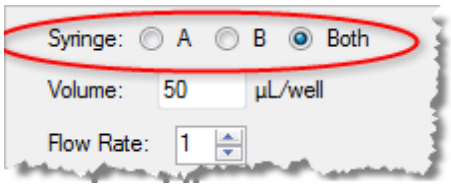


1536-well plates require two screens to show all 48 columns. Select the → key to toggle between the two screens to select the columns to dispense to.

1536F - 1536-well Flanged Plates

Important: Crashes can occur! Remove unused manifolds when dispensing to 153F plates.

To prevent an unused Peri-pump cassette or Syringe manifold from colliding with the plate flange during dispensing:



- use **both** Syringe manifolds simultaneously, and,
- unload the Peri-pump cassette when it is not being used;
- alternatively, remove the Syringe manifolds while using the Peri-pump;
- limit protocols to one dispenser only, either the

Syringe or Peri-pump, i.e. run multiple protocols on a plate when the assay requires using more than one device.

▪ **See About Wash Processing Patterns on page 105** for plate washing.

To change the plate map (selected columns):

1. When defining a protocol, press Enter until the plate map screen is shown.
2. ◂ ▸ Use the arrow keys to move the cursor to the column/sector you want to change. The cursor underlines the currently selected column and its number is shown in the display. (In the top example, column 2 is currently selected.)
3. Press the **Options** key to toggle between filling the column or not. When the image of the column is filled it will be dispensed to. Conversely, when the column image is blank or unfilled, the column will not be dispensed to.
4. Press **Enter** to save the settings and continue.

Skip "rows" when Peri-pump Dispensing

The Peri-pump offers an additional way to limit dispensing to a plate: **See Dispense Processing Patterns on page 108** .

Wash Step Format: Plate or Sector

When washing a high-density plate you can choose between two processing methods, Plate or Sector:

- **Plate format** performs each wash cycle to the entire plate before it starts the next cycle.
- **Sector format** performs the entire wash step to each sector of the plate before it moves to the next sector. A sector is defined by the manifold's footprint:
  - 96-tube manifolds process 384-well plates in 4 evenly spaced sectors (quadrants);
  - 192-tube manifolds process 384-well plates in two sectors: even and odd numbered columns.
  - 128-tube aspirate manifolds process 1536-well plates in 12 sectors;
  - 32-tube dispense manifolds process 1536-well plates in 48 sectors or 24 sectors when the manifolds are used simultaneously.

 Use sector mode when you are concerned about the fluid drying out before the procedure is completed. To learn more **See About Wash Processing Patterns on page 105**

How to shake the plate

These instructions are for keypad control.

The shake command is tied to the **Soak** option. These instructions apply to soaking or incubating the plate at room temperature, as well as shaking.

There are two ways to specify a shake period:

- **During a wash cycle**: To shake (and/or soak) in between every wash cycle, select **OPTS** when defining Wash Step Parameters and then select **MIDCYC**, and follow the prompts to specify parameters.
- **Shake step**: create a protocol to shake the plate, it can be a one-step, shake-only protocol, or you can **ADD** a Shake step before or after another step.

▪ **See Create or Edit a Protocol (Keypad Only) on page 89**

▪ Soak is not the same as "submerge" the tips. You must define a prime step or use the AutoPrime feature to soak the tips in the priming trough.

▪ Soak is equivalent to incubating the plate at room temperature or delaying the protocol.

▪ Shaking and soaking the plate is also an option when defining a Wash step.

How to enter negative numbers (Keypad only)

Some protocol parameters, like Horizontal Dispense Position (X-axis), require inputting a negative number to improve performance.

To enter a negative value:

1. Using the number pad, start at 00 (zero) and press **Shift +Options** to display the minus sign.
2. Use the number pad to enter the desired value. The minus sign will remain, making it a negative value.

Using LHC to Control the EL406 Washer Dispenser

BioTek's Liquid Handling Control (LHC) software, which works with the EL406 Interface Software (IS), is a more graphically rich way to design protocols and control the instrument. The EL406 must be attached to and communicating with your personal computer (PC) for the LHC to function.

- The keypad must be displaying its **Main Menu** for the LHC to communicate with it.

Predefined Protocols

BioTek provides predefined protocols for maintenance routines, instrument qualification, and other purposes like serial dilutions.

 Click the **Open** button and locate the **EL406** folder to open a predefined protocol.

Learn how to Customize the Predefined Protocols on page 67 for your lab.

Communications Port

Click the Port link in the main screen

The LHC needs to know the COM Port - Communications Port: USB or Serial component on the instrument used to connect the washer-dispenser to the computer.

- Make sure the EL406 is connected to the computer, turned on, and not busy.

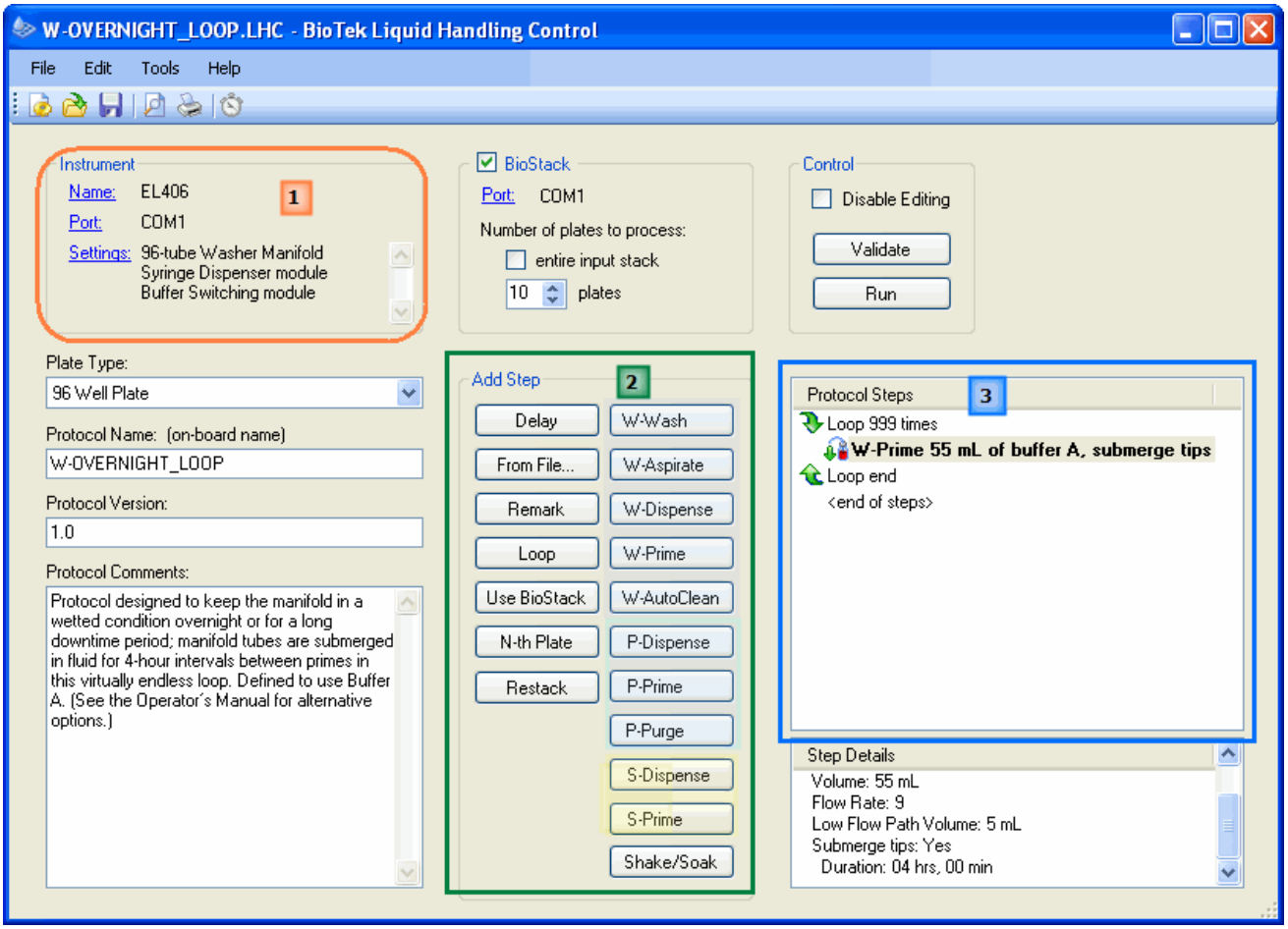
- Learn more **About COM Ports** in the **LHC Installation Guide** or select **Help>Help Topics**.

Click **Test Communications** after entering the number to verify its accuracy. The LHC will display a message.

If communication is unsuccessful:

- **Check the cabling**: make sure you're using a new/undamaged BioTek-supplied cable and it is properly inserted into the instrument's USB or serial port.
- **Turn on the washer-dispenser**: make sure the instrument is on and not busy processing a plate, running AutoPrime, or performing a system test, for example.
- **Retry**: contact BioTek TAC if you are still unable to establish communication between the instrument and the PC.

Introducing the LHC Workspace



1 Instrument Settings: Click the **Name** link and select your instrument.

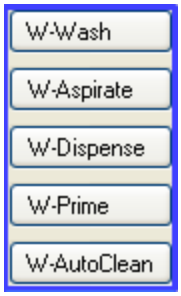
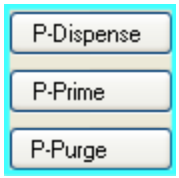
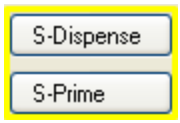
2 **EL406 Steps on the facing page**

3 Define a Protocol: select **Help>Help Topics.**

EL406 Steps

Because the EL406 offers three devices in one instrument: a microplate washer, a peristaltic pump dispenser called the Peri-pump, and dual Syringe dispensers, there are action buttons for each component. You will only see buttons that correspond to the devices and options available on your configuration of the instrument (as defined in Target Instrument Settings.)

Click the action button and define the parameters to add that step to the protocol:

	**W**- for steps performed by the **Washer**.
	**P**- for steps performed by the **Peri-pump** dispenser.
	**S**- for steps performed by one of the **Syringe** dispensers, when installed.
	Shake and soak are device-independent.

Each step is executed sequentially. You can combine steps performed by different devices in one protocol. For example, a W-Wash can be preceded or followed by a P-Dispense. Invalid combinations of steps will be identified when you press the **Validate** button.

See How to define a Protocol (LHC only) on page 102

LHC Protocols

BioTek provides predefined protocols for maintenance routines, instrument verification, and general samples for common applications like serial dilutions.

Review the **Predefined Protocols on page 69**

Customize the Protocols

Typically, you must modify the predefined protocols to match your instrument configuration and to meet your assay requirements.

Instrument Settings: In addition to action steps, every protocol file contains instrument settings, including COM port, manifold type, and so on. Edit the protocol to match your instrument's COM Port and other configuration details:

- Customize the Predefined Protocols on page 67
- **Power Users**: If you create protocols for multiple instruments or for other LHC users, read this more detailed description of how the EL406 validates a protocol to be run on a specific instrument.

> ▪ **Recommended**: Before changing a predefined protocol, select **File>Save As** and give it a unique name. This practice preserves the custom protocol in the case of a future upgrade.

File Location

The LHC installs the protocols in the Windows Common Applications Data Folder:

- Windows® XP: C:\Documents and Settings\All Users\Application Data\
- Windows® Vista™ and Windows 7: C:\ProgramData\

The file location path continues:

[CommonAppDataFolder]**BioTek\Liquid Handling Control *v.#*\Protocols\EL406**

Three folders are provided:

- **\Maintenance**: the recommended daily and periodic maintenance routines;
- **\QC**: some of the quality control or performance verification procedures;
- **\Samples**: examples of common applications, including washing 96- and 384-well plates, performing serial dilutions, and a cell wash protocol.

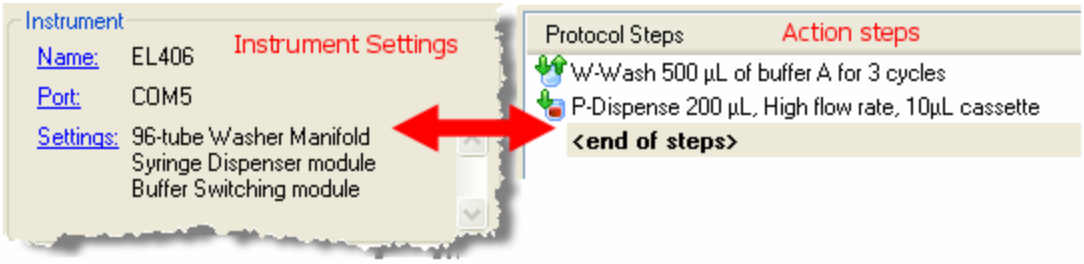
LHC Protocols Explained

Prerequisite

This discussion about EL406 protocols will be easier to follow if you are already familiar with the LHC. Read "Understanding the LHC" in the Help.

Protocol Files

In addition to the "Protocol Steps" (the actions you tell the EL406 to perform to process plates), each protocol file contains "Instrument Settings."



The LHC must know an instrument's settings in order to create a protocol that will run on that instrument. This virtual "Target Instrument Settings" feature lets you write protocols when the instrument is not connected to your computer.

Generally, and especially when you are managing only one instrument, the best practice is to always match the instrument settings to your instrument. (Select "Get actual settings" from the connected instrument. Unless the instrument is not connected to the computer, then, you must specify the settings.)

The "Instrument Settings" stored in the protocol file include the COM port and configuration details like the type of manifold/dispense cassette installed, the presence of a Peri-pump or Syringe dispenser, and other details that are critical to controlling the instrument.

The LHC keeps track of the last-used COM port for an instrument type. For example, when an EL406 runs a protocol, the LHC logs the COM port used and the next time an EL406 is used, the LHC applies the same COM port setting. You can disable this feature by defining your Ports preference: select **Tools>Preferences>Ports**.
To correct the COM port for the current protocol, click the **Port** link and use the drop-down list to select the correct value. The LHC stores the COM port value in the protocol file.

Managing Multiple Instruments

The target instrument settings feature is useful for those managing multiple instruments. In addition to the flexibility of being able to create protocols for non-

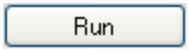
connected instruments, you can create and save an instrument settings file for each of your liquid handlers, another time saver.

Protocols are considered valid when an instrument can successfully perform the protocol. The LHC will run a protocol even when the instrument settings do not match the physical configuration of the instrument. For example, a protocol with instrument settings that include Buffer Switching can be run by an instrument without Buffer Switching when none of the steps actually call for different buffer valves, i.e. all steps use the same buffer.

Similarly, an EL406 that does not have the Syringe dispenser installed can run protocols with instrument settings that include the Syringe, as long as the protocol does not include any Syringe dispenser steps. This flexibility is useful when you are designing protocols for multiple instruments.

Validate versus Run

Validate checks the action steps against the protocol's Target Instrument Settings.



Run talks to the instrument to check the action steps against the instrument's onboard settings.

Validate will catch errors when the Instrument Settings have been changed after the protocol steps have been defined and there is a mismatch. **Run** performs a similar validation before executing the protocol. Errors are not reported unless the steps cannot be performed.

Target Instrument Settings

- For LHC users only.

Click the **Settings** link in the main workspace

Actual Instrument or Simulated Instrument? That is the question for the Target Instrument Settings dialog.

When the EL406 is:

- connected to the computer: it is best to "Get (the) actual settings" from it;
- not connected to the computer: you must define the settings.

One vs. Multiple Instruments

- If you are running only one instrument, always "Get the actual settings" to identify the exact configuration of your EL406 to ensure it can successfully perform the wash and

dispense protocols.

- If you are managing multiple BioTek instruments (or one instrument with multiple configurations): you can create and save a "settings file" for each instrument to help create protocols for that instrument when you are not connected to it. See below.

The "instrument settings" tell the LHC what the instrument can do, e.g. fill a 384-well plate or not. It is impossible to create a protocol without this information. Read this to understand the correlation between the Target Instrument Settings and the protocol.

Get settings from:

- **Instrument**: BioTek configures and tests the EL406 at the factory before shipping it. If you have not changed the instrument's onboard settings, you can safely click the **Get** actual settings now link to upload the correct settings from the instrument.
- **Settings file**: If you have previously saved the instrument's settings to a file (using the **Save** link), click this link to import them.
- **This screen**: manually define the instrument's settings and click **OK**. This option does not affect the instrument's onboard configuration settings. It lets you define protocols for an instrument with the specified components.

Configured with:

Select the appropriate devices to identify your instrument's components:

- **Washer Manifold**:

96-tube Single	96-well plates only
96-tube Double	96-well and 384-well plates
192-tube	384-well plates only
128-tube Aspriate	1536-well plates only

- **Syringe Dispenser**: when a dual syringe pump dispenser is installed, identify the dispense manifold type:
  - Non-Autoclavable: the black plastic casing of the non-autoclavable syringes are easy to distinguish from the glass and stainless steel autoclavable syringes. This model only supports the 8-channel and 32-channel manifolds.
  - Autoclavable: glass and stainless steel autoclavable syringes.

8-tube	Double manifold with two sets of 8 tubes.
16-tube	16-channel manifolds.
16-7 tube	16-channel with tubes angled 7°.
32-tube	1536-well plates only;

	Model: Large Bore (LB) or Small Bore (SB)

- **Peri-pump Dispenser**: when the instrument is equipped with a peristaltic pump for 8-channel dispensing.
- **Buffer Switching**: when this external valve module is installed for automatically switching wash buffers/reagents for either the washer or Syringe dispenser.
- **Vacuum Filtration**: when the instrument is equipped with the special carrier for washing filter-bottom microplates.
- **Ultrasonic Advantage**: when the instrument is equipped with the stainless steel reservoir and ultrasonic cleaning capability.

> ▪ Be sure to complete all the steps for Changing the Washer Manifold, when applicable.

Save Settings File

If you have multiple instruments or use one instrument in multiple configurations, you can create unique settings files for each configuration and save time when defining protocols for that configuration.

Click the **Save** link and use Windows' file-saving dialog to create a .SET file based on the currently-selected parameters. Then use the **Get** "settings from a previously saved" link to load the parameters.

How to define a Protocol (LHC only)

> ▪ For keypad instructions: Create or Edit a Protocol on page 89

In short:

- Select the Plate Type and assign a unique Protocol Name Limit the name to 16 alpha-numeric characters if you want to run the instrument using the keypad only, i.e. disconnected from the computer..
- Click a button in the Add Step area.
- Define the parameters for the step in the dialog that opens.
- Continue adding steps, if desired.
- Save the file and/or click **Run** to execute the protocol.

 Double-click a step in the protocol to open it for editing.
- Highlight a step and press **Delete** to remove it.
- Click and drag a step to change its sequence order.

Plate Types and Processing Patterns

Depending on the type of hardware installed on the instrument, e.g. manifold type, the EL406 can process several plate types. The default parameters for wash and dispense steps represent the optimal positioning of the hardware for the plate type. And, the aspirate and dispense heights and horizontal positions can be adjusted when necessary for special situations and to optimize assay performance.

- Review the Plate Types Table below for a listing of supported plates and their geometries;
- **See About Wash Processing Patterns on page 105** in the Help system or operator's manual.
- **See Dispense Processing Patterns on page 108** in the Help system or operator's manual.
- Review these instrument settings that may improve your work flow:
  - Plate Carrier Setting
  - Plate Clearance Setting
  - Peri-pump Dispense Pattern

Plate Types Table

Only the Peri-pump can process all plate types. Washer manifolds limit processing to plates with the same geometry. Only the 32-tube Syringe dispenser manifolds can dispense to 1536-well plates.

Plate Type	On board Name	Columns x Rows	Plate Height	Default Aspirate/ Dispense Height		EL406 sup-ported
			mm	Wash-er	Dis-pensers	
96 Well	96	12x8	14.35	29/121	336	✔
96 Deep Well	96D	12x8	41.50	335	929	
96 Half Well^	96H	12x8	14.20	120	332	
96 Mini Tubes	96MT	12x8	49.53	398	1105	
384 Well	384	24x16	14.22	22/120	333	✔

Plate Type	On board Name	Columns x Rows	Plate Height	Default Aspirate/ Dispense Height		EL406 sup-ported
			mm	Wash-er	Dis-pensers	
384 Deep Well	384D	24x16	44.08	355	986	
384 PCR²	384P	24x16	9.50	2/83	230	✔
1536 Well	1536	48x32	10.41	42/-	250	✔
1536 Flanged‡	153F	48x32	10.26	13/-	196	✔

Peri-pump Only Plates					
Plate Type	**Onboard name**	**columns x rows**	**Plate Ht. (mm)**	**Dispense Ht. (steps)**	**Tubes/ well**
6 Well	6	3x2	20.20	464	4*
12 Well	12	4x3	20.20	464	2*
24 Well	24	6x4	20.50	470	2
48 Well	48	8x6	20.10	461	1*

^ Only available for 8-channel manifolds
‡ Only 1536 Flanged (153F) plates have a "flange height" greater than zero. These plates require special handling.

- ***Important**: when dispensing to 6-, 12- and 48-well plates some dispense tubes must be removed from the fluid supply vessel. **See Handling Special Plates and Mini-tubes on page 110**

Plate Geometry Diagram

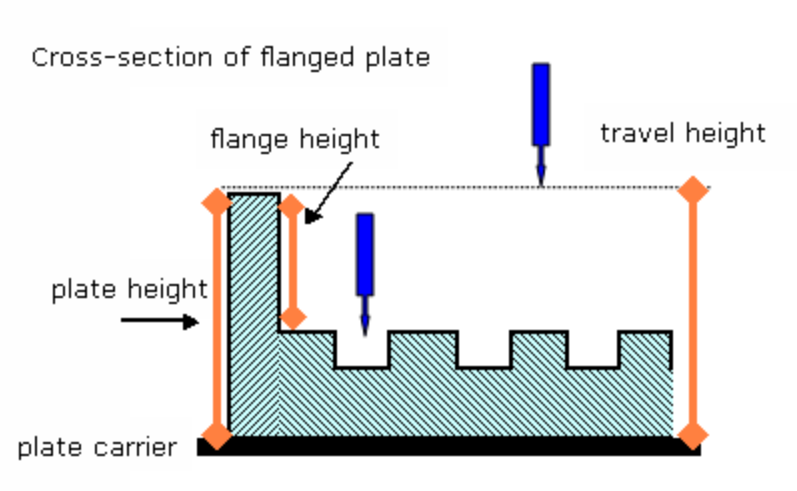


Plate height = physical measurement

Default Dispense Height = (plate height - flange height + 1.0 mm)

Travel Height = (plate height - flange height + Plate Clearance)

- If Dispense Height > Travel Height (greater than), the travel height is changed to match the dispense height.

About Wash Processing Patterns

When using a 96-tube manifold to process a 96-well plate, all wells are processed simultaneously. To process 384- and 1536-well plates, a processing pattern is needed, which also provides the ability to process the plate partially. Some portions of the plate can be left untouched or panels can be defined, i.e. multiple assays can be run on the same plate.

The processing pattern is determined by the hardware's footprint.

32-Tube Syringe Dispenser Manifolds

One 32-tube dispense manifold addresses one column at a time. When both 32-tube manifolds are used simultaneously, they align with every 5th column, in a pattern like this:

Columns													
1				5				9				13	
	2				6				10				14
		3				7							
			4				8			and so on...			

Columns 1 and 5 are dispensed to first, then columns 2 and 6, 3 and 7, and so on.

During a wash step the dispenser mirrors the pattern defined for the aspirate manifold, dispensing to the same columns in the same order they were aspirated from.

96-tube wash pattern for 384-well plate

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
A	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B
B	C	D	C	D	C	D	C	D	C	D	C	D	C	D	C	D	C	D	C	D	C	D	C	D
C	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B
D	C	D	C	D	C	D	C	D	C	D	C	D	C	D	C	D	C	D	C	D	C	D	C	D
E	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B
F	C	D	C	D	C	D	C	D	C	D	C	D	C	D	C	D	C	D	C	D	C	D	C	D
G	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B
H	C	D	C	D	C	D	C	D	C	D	C	D	C	D	C	D	C	D	C	D	C	D	C	D
I	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B
J	C	D	C	D	C	D	C	D	C	D	C	D	C	D	C	D	C	D	C	D	C	D	C	D
K	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B
L	C	D	C	D	C	D	C	D	C	D	C	D	C	D	C	D	C	D	C	D	C	D	C	D
M	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B
N	C	D	C	D	C	D	C	D	C	D	C	D	C	D	C	D	C	D	C	D	C	D	C	D
O	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B
P	C	D	C	D	C	D	C	D	C	D	C	D	C	D	C	D	C	D	C	D	C	D	C	D

To process a partial plate, you must select the sector or sectors you want to process:

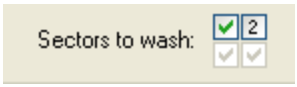
Checkmarks show the sectors to be processed. Numbers show potential sectors that are currently unselected and will not be washed. The 96-tube manifold processes the plate in four sectors.

A	B
C	D

192-tube wash pattern for 384-well plate

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
A	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B
B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B
C	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B
D	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B
E	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B
F	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B
G	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B
H	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B
I	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B
J	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B
K	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B
L	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B
M	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B
N	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B
O	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B
P	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B

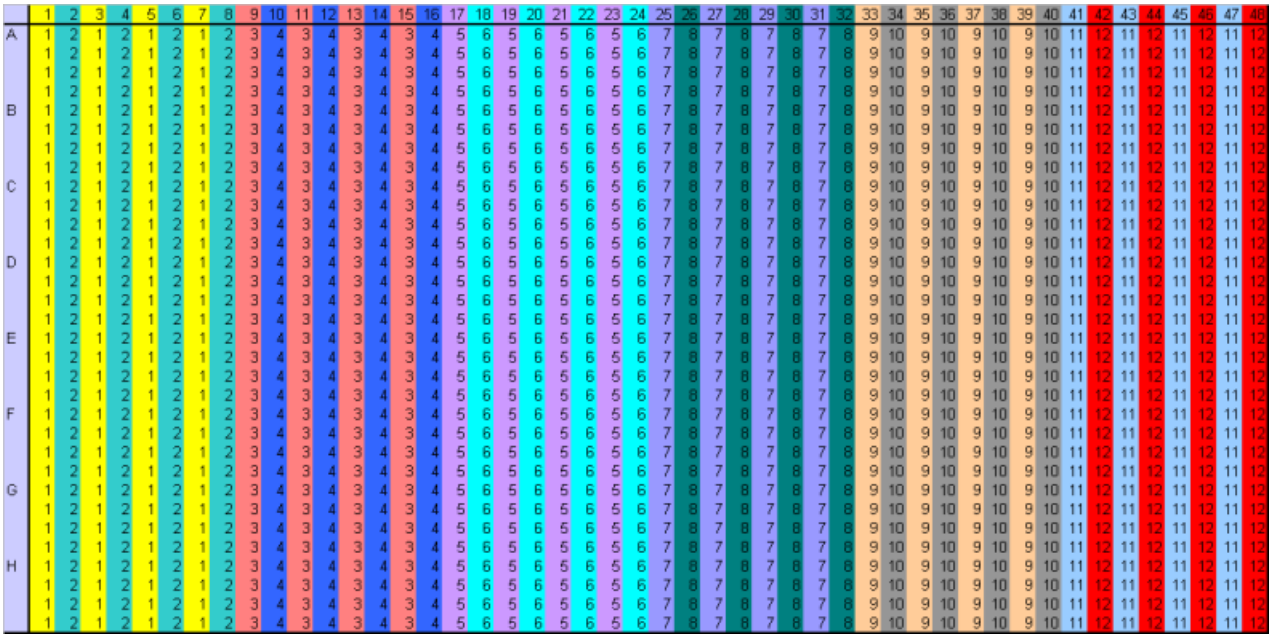
To process a partial plate, you must select the sector you want to process:



Checkmarks show the sectors to be processed. Numbers show potential sectors that are currently unselected and will not be washed. The 192-tube manifold processes the plate in two sectors.

A	B

128-tube wash pattern for 1536-well plate



The 128-tube aspirate manifold processes 1536-well plates in 12 sectors. When you select the columns to process for a partial plate, they correspond to these sectors.

or

In a wash step, click the Columns link: **All** or **Partial** to define a partial plate.

Selections correspond to the 12 sectors.

In a wash step, the dispenser mimics the pattern of the aspirate manifold, i.e. dispenses to the same columns in the same order.

Dispense Processing Patterns

When dispensing to high-density plates, 384- and 1536-well, the 8-channel Peri-pump and the various Syringe dispensers employ a dispensing pattern to fill the plate. The dispenser's footprint determines the pattern required to fill a plate and provides the ability to process a partial plate, skipping some sections.

- Washing Plates: Learn about processing patterns.

Columns

To skip an entire column toggle the radio button off. With this feature you can dispense to odd columns in one dispense step, change fluids or use another dispenser and dispense to even columns in another dispense step.

Rows

The Peri-pump offers another way to control the dispense pattern to high-density plates. See Peri-pump Dispense Patterns.

32-Tube Syringe Dispenser Manifolds

One 32-tube dispense manifold addresses one column at a time. When both 32-tube manifolds are used simultaneously, they align with every 5th column, in a pattern like this:

Columns													
1				5				9				13	
	2				6				10				14
		3				7							
			4				8			and so on...			

Columns 1 and 5 are dispensed to first, then columns 2 and 6, 3 and 7, and so on.

During a wash step the dispenser mirrors the pattern defined for the aspirate manifold, dispensing to the same columns in the same order they were aspirated from.

Peri-pump Dispense Pattern

When addressing high density plates, 384- and 1536-well, the 8-channel cassette moves in the pattern described here to fill the plate. With Advanced Dispense Options, you can skip certain rows and columns.

Because the Peri-pump cassette addresses every column during a dispense, you can specify which columns to skip during a dispense step.

384-Well Plate

Rows: You can skip one of the two "rows" sections:

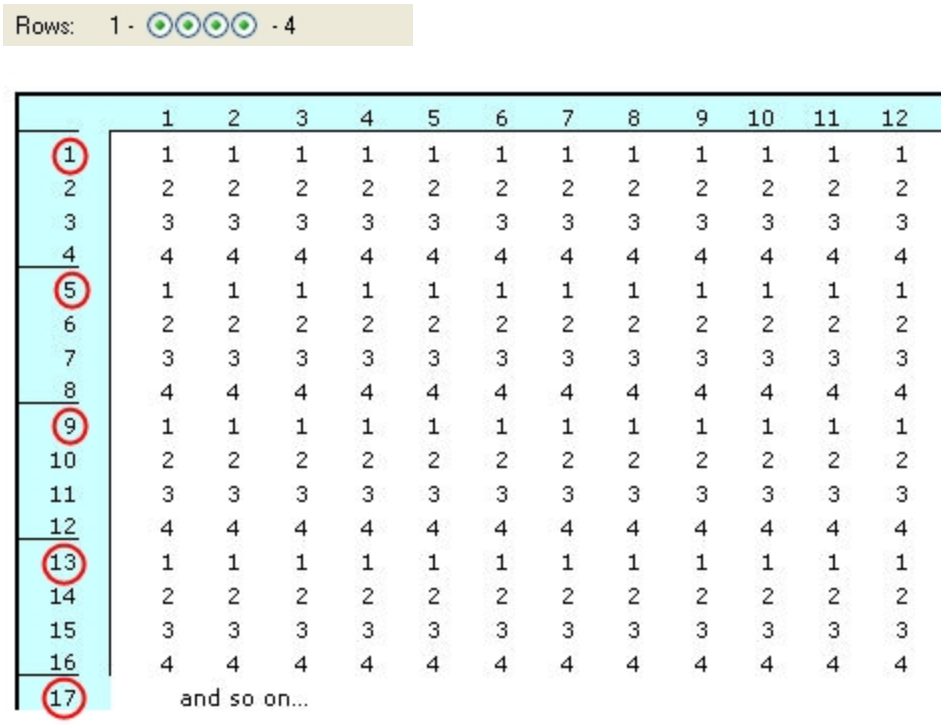
		1	2	3	4	5	6	7	8	9	10	11
1	a	1	1	1	1	1	1	1	1	1	1	1
2	b	2	2	2	2	2	2	2	2	2	2	2
3	c	1	1	1	1	1	1	1	1	1	1	1
4	d	2	2	2	2	2	2	2	2	2	2	2
5	e	1	1	1	1	1	1	1	1	1	1	1
6	f	2	2	2	2	2	2	2	2	2	2	2
7	g	1	1	1	1	1	1	1	1	1	1	1
8	h	2	2	2	2	2	2	2	2	2	2	2
9	i	1	1	1	1	1	1	1	1	1	1	1
10	j	2	2	2	2	2	2	2	2	2	2	2
11	k	1	1	1	1	1	1	1	1	1	1	1
12	l	2	2	2	2	2	2	2	2	2	2	2
13	m	1	1	1	1	1	1	1	1	1	1	1
14	n	2	2	2	2	2	2	2	2	2	2	2
15	o	1	1	1	1	1	1	1	1	1	1	1
16	p	2	2	2	2	2	2	2	2	2	2	2

When the 8-channel cassette addresses a 384-well plate, it first dispenses to odd numbered rows, and then dispenses to even numbered rows. With this feature you can dispense to odd rows in one dispense step, change fluids or use another dispenser and dispense to even rows in another dispense step.

In addition, you can combine a selection of columns with one of the two row sections (odd or even) to define a complex distribution pattern.

1536-Well Plate

Rows: You can skip up to three "rows" sections:



When the 8-channel cassette addresses a 1536-well plate, it first dispenses to rows 1, 5, 9, 13, 17, 21, and 25. The next section of rows dispensed to includes 2, 6, 10, 14, 18, 22, 26, and 30. And so on, creating 4 sections of rows.

Handling Special Plates and Mini-tubes

Note: Also see the Plate Types Table on page 103.

Peri-pump Only Plates

The **Peri-pump** supports several plate types, but vessels with fewer than 8 rows require special handling. Some adjustment of or consideration of how the 8-channel dispense head will address the plate is needed.

The Peri-pump's **dispense volume is per tube or channel**, not per well. This is the most important fact to consider when using these special plates. The volume defined in a Quick Dispense or protocol is the amount each tube will dispense to the well.

When you use multiple tubes to address a well, specify the desired volume with this multiple in mind. For example, two tubes can address each well in a 24-well plate, so the "defined" volume must be half the desired volume. As always, be sure

to also consider the Peri-pump's optimal performance settings when designing the dispense protocol, i.e. full aliquots are more accurate than fractions of an aliquot.

Another tool to consider using with special plates is the X- or Y-axis Dispense Position setting. You may be able to use it to aim the dispense tubes to a certain region of the well.

6-, 12-, 24-, and 48-Well Plates

BioTek recommends experimenting with different dispense-tube-to-well configurations when using these special plates. For some plates, multiple tubes can dispense to a single well. Conversely, dispense tubes can (and sometimes, must) be removed from the supply vessel or from the cassette to prevent them from missing the wells.

Testing at BioTek found the following capabilities:

Plate Type	Columns/Rows	Tubes per Well	Tubes removed
6 Well	3 x 2	3 or 4	(4 and 5) or 0
12 Well	4 x 3	2	3 and 6
24 Well	6 x 4	2	0
48 Well	8 x 6	1	3 and 6

- **6-well plates** with 2 rows: three or four tubes can fit in the wells. Remove tubes 4 and 5 to use just three tubes per well. Fewer tubes may be preferred to preserve cells in certain assays.
- **12-well plates** with 3 rows: two tubes can fit in the wells, so 6 tubes will be used. Remove tubes 3 and 6.
- **24-well plates** with 4 rows and large enough wells to support two tubes are the easiest of these special plates. All 8 tubes can be used, 2 per well, for standard microplates. Exception: the special 24-vial rack, PN 7212058, requires removing the even numbered tubes from the cassette or fluid supply.
- **48-well plates** with 6 rows: requires two tubes, 3 and 6, to be removed.

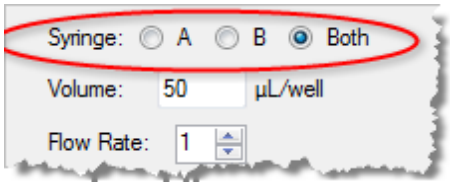
> ▪ Remember, when defining a run: **dispense volume is per tube**, not per well. Divide the desired volume by the number of tubes in a well.

If you regularly use 6- or 48-well plates, which require dispense tubes to be removed from the fluid supply before dispensing, you should consider dedicating certain cassettes for the purpose. Removing the unused tubes from the cassette, rather than from the fluid supply, will preserve them for future use and make the cassette easier to handle. Review the instructions to Replace Peri-pump Dispense Cassette Tubing on page 210.

1536F - 1536-well Flanged Plates

Important: Crashes can occur! Remove unused manifolds when dispensing to 153F plates.

To prevent an unused Peri-pump cassette or Syringe manifold from colliding with the plate flange during dispensing:



- use **both** Syringe manifolds simultaneously, and,
- unload the Peri-pump cassette when it is not being used;
- alternatively, remove the Syringe manifolds while using the Peri-pump;
- limit protocols to one dispenser only, either the Syringe or Peri-pump, i.e. run multiple protocols on a plate when the assay requires using more than one device.

To achieve the best dispense performance when processing 1536-well flanged plates, the EL406's default protocol settings specify a low dispense height to position the dispense tubes as close as possible to the wells. When the dispense arm holds multiple dispense manifolds, the manifolds not addressing the wells can collide with the plate flanges during a dispense.

Change the Plate Clearance Setting (Keypad)

The Plate Clearance setting adds the specified (input) value to the travel height for the selected plate type, i.e. it adds this number to the "Plate Height" value cited in the Plate Types Table. The manifold rises to this height to move from one column to the next and whenever repositioning is needed. This setting does not affect dispense and aspirate heights.

Use this setting to accommodate plates that are slightly taller than standard plates to make sure the manifolds rise high enough above the plate to prevent crashes when the plate carrier moves.

Note: Enabling the Magnet Adapter Height affects the travel height, as well as dispense and aspirate heights. The Magnet Adapter Height increases the respective values of all height parameters. Generally, it is best to keep the default setting for Plate Clearance when using the Magnet Adapter Height setting.

To change the setting using the keypad:

1. Press **Setup Menu** (button in center of keypad).
2. Select → and **ADVANC** then **PLTCLR**.
3. Enter an offset value in millimeters (mm).

Change the Magnet Adapter Setting (Keypad)

When performing magnetic bead assays/biomagnetic separation, this offset setting is a real time saver. It increases the height or Z-axis for all processing options to accommodate the increased height of the plate when the magnet is installed.

This setting eliminates the need to modify individual protocol parameters to adjust the dispense and aspirate heights and enables Quick Dispense and Quick Wash (without adjustments) when the magnet is used. It applies the specified height value as an offset to all relevant steps.

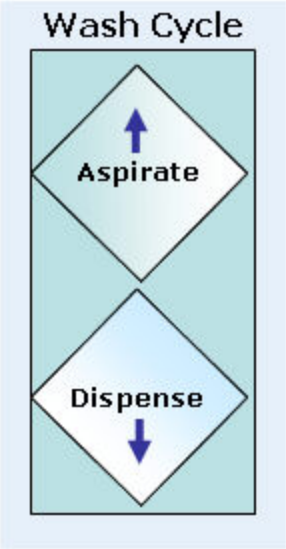
 Custom or non-standard microplates and special adapters for labware may benefit from this setting, too. If a vessel's height has been its only limitation to processing with the EL406, i.e. the vessel's geometry permits the manifold tips to successfully address its wells, this setting can be used to simplify protocol development.

See Determine Magnet Height Offset on page 130.

After determining the offset value, change the setting using the keypad:

1. Press **Setup Menu** (button in center of keypad).
2. Select → twice and then **MAGHT**.
3. Select **Yes**. This setting tells the instrument if the magnet adapter is currently installed or not. It lets you retain the height offset value while turning off its application, i.e. when you are no longer using the adapter, change this setting to No. The EL406 will remember the offset value.
4. Enter an offset value in millimeters (mm).

About the EL406 Wash Step



Minimally, a wash step includes an aspirate step followed by a dispense step. This is a **wash cycle**.
The default definition of a wash step includes 3 cycles, followed by a final aspiration to evacuate the wells.
It is important to prime the washer manifold before running a wash. You may also want to include a Pre-dispense or tip prime (over the priming trough) to correct for evaporation and other minor fluid loss to normalize the tips.
About the hardware: The 96- and 192-tube wash manifolds perform both steps, aspirating and dispensing fluid to and from 96-well and 384-well plates. Models that support 1536-well plates have an 128-tube aspirate manifold and two 32-tube syringe-pump dispensers to wash the plate.

Recommendations for Priming the Washer

	When tubes are empty		When changing fluid	
With Buffer Switching	Prime Volume	Low Flow	Prime Volume	Low Flow
	750 mL	50 mL	900 mL	100 mL
Without Buffer Switching	Prime Volume	Low Flow	Prime Volume	Low Flow
	360 mL	50 mL	400 mL	100 mL

Recommendations for 1536-well hardware

Our experiments using the 128-tube aspirate manifold and the 32-tube Syringe dispenser manifolds yielded the following recommendations. "Your mileage may vary": experiment with the parameters and settings to determine the optimal values for your assays.

- High-throughput: to speed up processing try these settings:

Aspirate Travel Rate	6 CW
Dispense Flow Rate	5

- Improve Accuracy: set the Dispense Flow Rate to 2.
- Improve evacuation for the smallest residuals: use a standard Aspirate Travel Rate, not a CW (cell wash) rate.
- Retain fluid in the wells, increase residuals: increase the aspirate height, Z Position. For example, in our testing, 56 steps, instead of 40, left 3 µL deionized or distilled water in each well.

 Remember to keep the dispense manifolds wet when not in use: soak the tubes in the priming trough inserts with **AutoPrime**, for example.

Add a secondary aspiration to a wash cycle

When too much residual is left in the wells during a wash cycle, you can add a secondary aspiration to reduce it. Secondary aspiration can be performed immediately after each aspiration in a wash cycle and/or after the final aspiration step. It is not available for 1536-well plates.

- Secondary aspiration is also called Crosswise aspiration because it is typically performed in a different location within the well. Reposition the tubes by defining different X-, Y-, and/or Z-axis positions when enabling the secondary aspiration for the most effective evacuation of the wells.

Edit the Wash step or stand-alone Aspirate step to add a secondary aspiration:

LHC	Keypad
1. Double click the step in the protocol to open it for editing. 2. Click **Advanced Options** (for Aspirate) and enable Perform Secondary Aspirate. 3. Apply a second aspiration to the Final Aspirate, also, for best evacuation results. 4. Optionally, adjust the position of the manifold as it addresses the wells.	1. Edit the Wash step or stand-alone Aspirate step: **Define>Edit>**select protocol, and if necessary press Options to scroll to the desired step. 2. Select **ASPIR** and press Enter until Secondary Aspirate is offered. And/or select **OPTS>POST** to add a second aspiration to the Final Aspirate. 3. Select **YES** to perform a second or crosswise aspiration. 4. Specify a crosswise position for the manifold as it addresses the wells.

For example, you may want to adjust the X- or Y- axes to get to hard-to-reach areas of certain types of wells. Or, you may want to lower the height (reduce the Z-axis), to better evacuate fluid.

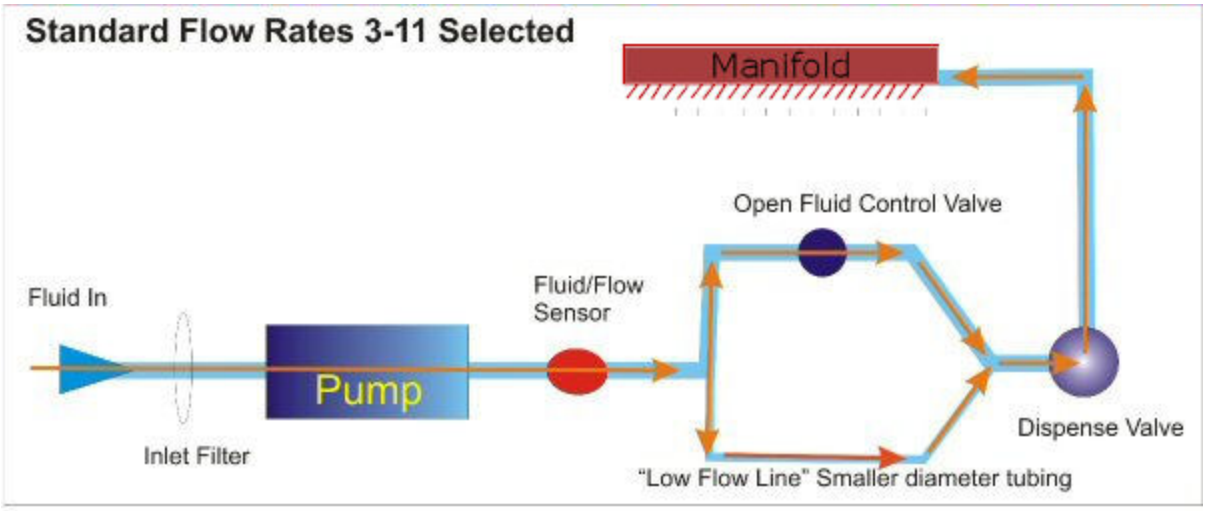
Cell Wash

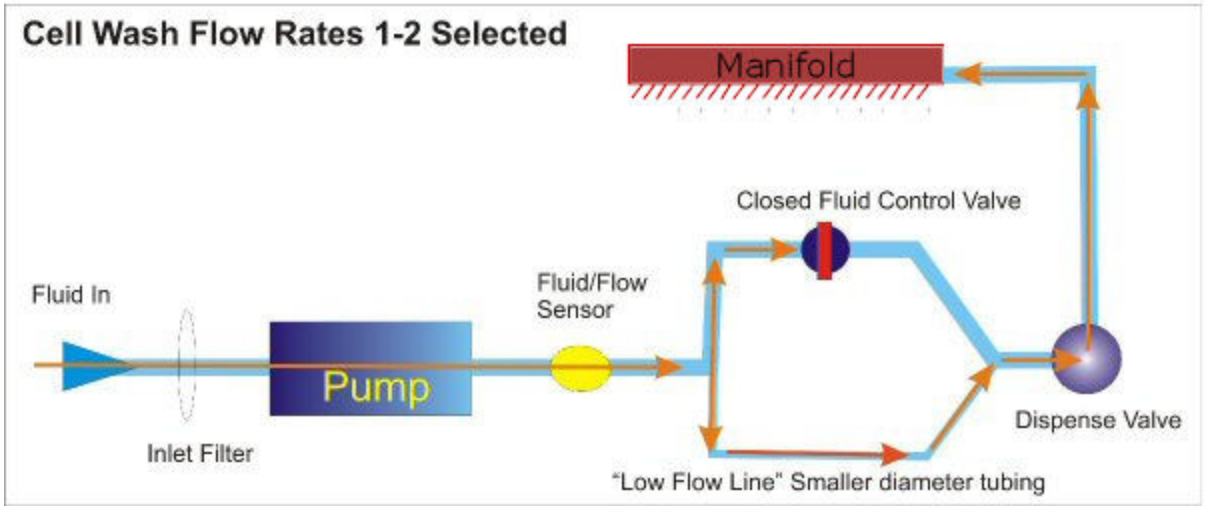
- Not all EL406 models support cell wash assays. The dual 96-tube wash manifold must be installed.

See how to **Define a Cell Wash Protocol on next page.**

Low-flow Fluid Path

The EL406 supports cell-based assays that require the addition and removal of buffer solution without disrupting the cells in the wells of the microplate. Cells are often dislodged when fluid is dispensed at too high a pressure and lost during subsequent aspiration of the fluid from the well unless counter measures are taken. The EL406 is equipped with a low-flow fluid path that provides a "cell wash" alternative for cell-based assays.





The low-flow tubing is used during a wash step when the Flow Rate is set to 1 or 2. It dispenses fluid to the wells slowly enough to avoid damaging the cells. Note that the low flow line is always open, i.e. some fluid flows through the tubing during normal dispenses. For this reason, priming the low flow tubing is recommended for all Prime steps.

Additional Techniques

Delay Aspiration: Also critical to cell-based assays is delaying aspiration to allow the slower dispense process to finish before beginning fluid removal from the well. This option, offered as part of the dispense step, is called Delay Start of Vacuum.

Adjust the Aspirate Travel Rate and Aspirate Height: when defining the aspirate step select one of the specially designed travel rates that minimize turbulence in the wells. Increase the aspirate height to leave more residual fluid in the wells to protect the cell layer. Also, consider using a secondary aspiration as described in the Cell Wash Strategies described below.

Adjust the Dispense Flow Rate, Height and Position: when defining the dispense step be sure to select one of the special Flow Rates that trigger use of the low-flow tubing, 1 or 2 CW. Also reposition the dispense tubes to aim the fluid at the side of the wells to further minimize turbulence. **See Cell Wash Strategies on the facing page**.

CW+ Dispense Manifold

As a result of BioTek's continuous improvement effort for liquid handlers, the washer dispense manifold has evolved. The dispense tubes of the improved manifold ensure fluid hits higher on the walls of the well, minimizing damage to the cells. "CW+ Dispense Manifold" is engraved on the top of these newer manifolds to make them easy to recognize. If you do **not** have one of these manifolds you may need to experiment with the Washer Settings (the CW+ Control) to improve the performance of your cellular assays. Contact BioTek to obtain this special cell wash manifold.

Next: Define a Cell Wash Protocol below.

Define a Cell Wash Protocol

Adjust the volumes recommended in this procedure to meet your specific needs.

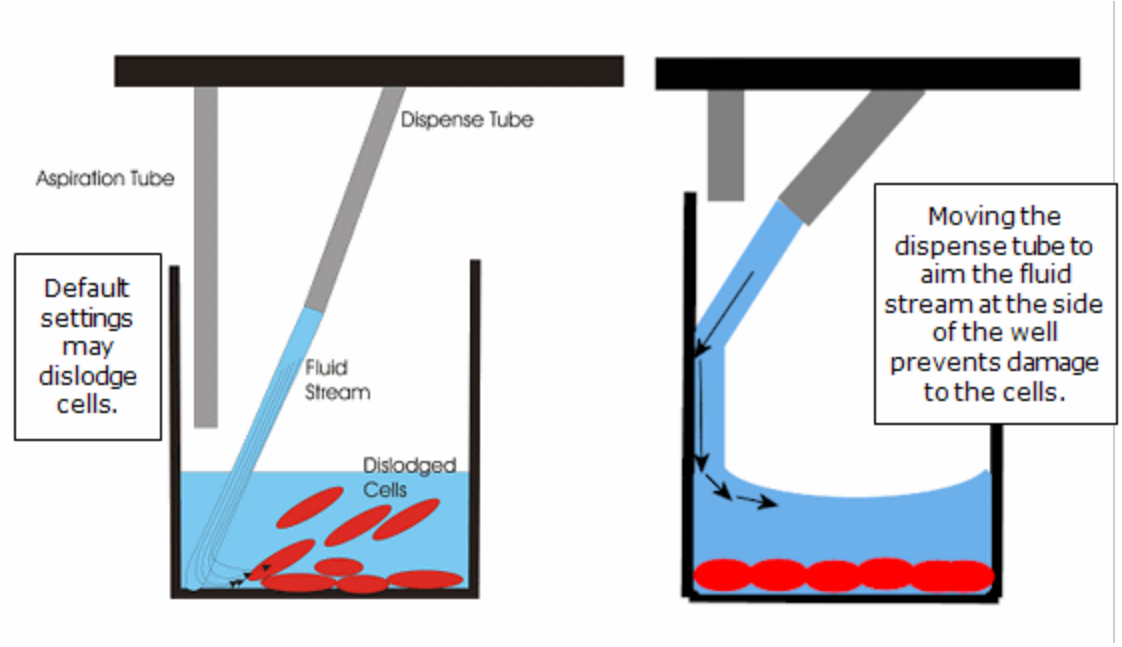
1. Create a new protocol.
2. W-Prime Add a **Prime** step, especially when the lines are empty or when changing fluids.
3. W-Wash Add a **Wash** step and define it as you normally would, except with these special parameters:
   - Set the Aspiration **Travel Rate** to **6 CW** and the Delay to **0**.
   - Set the Dispense **Flow Rate** to **1** or **2**.

- Click the Dispense **Advanced Options** link and enable the **Delay start of Vacuum** until sufficient fluid has been dispensed. For small dispense volumes, BioTek recommends setting the delay volume to equal your dispense volume.
- Reposition the dispense tubes to aim fluid at the side of the wells to reduce turbulence and change the aspirate height to increase the amount of residual fluid left in the well to protect the cell layer. **See Cell Wash Strategies below**.

Be sure to check the parameters for Final Aspiration.

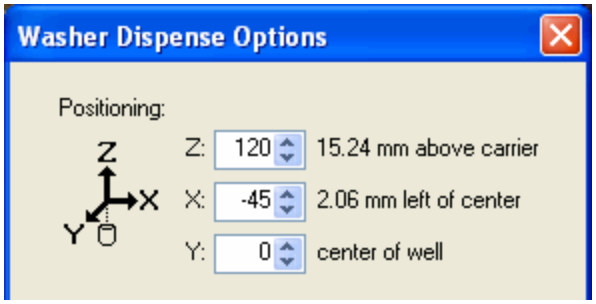
Cell Wash Strategies

To give you a starting point for optimizing your own assays, here are some recommendations for improving your cell wash assays:

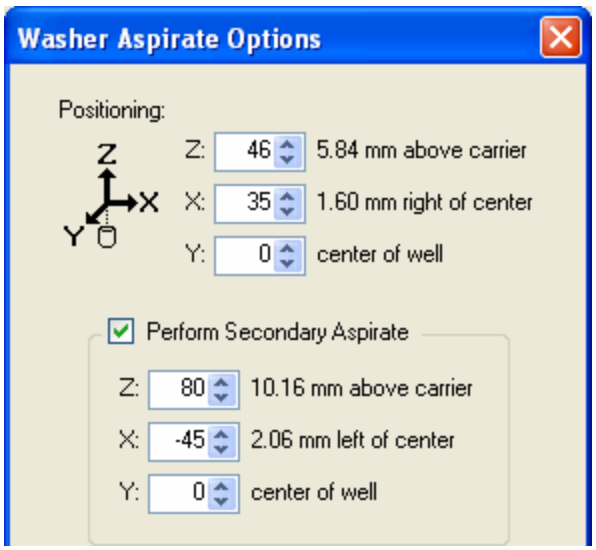


Repositioning the dispense and aspirate tubes helps minimize turbulence in the wells, preserving more cells. Using a standard 96-well Corning Costar plate, best results were achieved with these values:

Dispense Step Settings:
Z - height = 120 steps
X - horizontal position = -45
Y - horizontal position = 0



Aspirate Step Settings:
Z = 46 steps
X = 35
Y = 0
Secondary Aspirate:
Z = 80 steps
X = -45
Y = 0
About 40 µL/well residual fluid is retained using these values.



For loosely adherent cells, the best performance was seen by increasing the aspirate height and using both a standard and secondary (or crosswise) aspiration. Moving the aspirate tubes from one side of the well to the other prevents a fluid stream from forming and dislodging the cells. Increased residual in the well means increased cell retention.

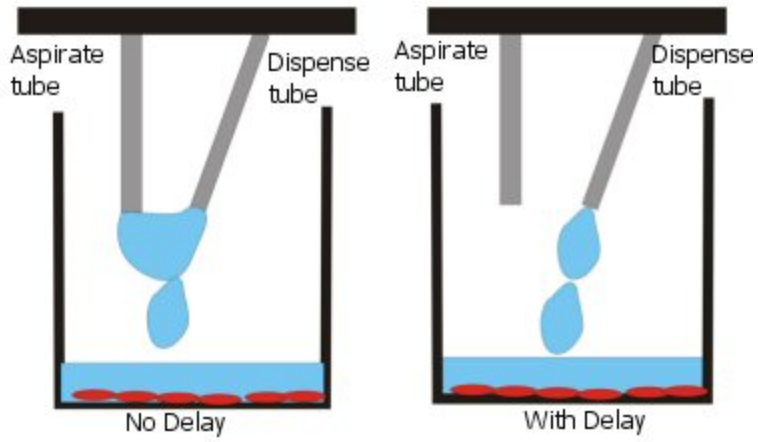
Best practice:

- Use the Adjust Utility to determine the optimal X, Y, and Z axis adjustments needed to best position the manifold above the wells during the wash routine.
- Test the protocol settings by running the protocol using only water and an empty plate before actually running your assay to make sure the fluid stream hits the wells as desired.
- Optimal cell wash performance is achieved with BioTek's special CW+ Dispense Manifold. "CW+ Dispense Manifold" is engraved on the top of these newer manifolds to make them easy to recognize. If you do **not** have one of these manifolds you may need to experiment with the Washer Settings (the CW+ Control) to improve the performance of your cellular assays. Contact BioTek to obtain this special cell wash manifold.

Delay Aspiration or Vacuum On Volume Control

Keypad Users: Edit the Wash or Dispense Step>Press Enter till Vacuum Delay Vol:

During regular plate washing, aspiration and dispensing occurs simultaneously. This allows "overflow" dispensing, because the fluid is aspirated before overflowing the plate. But, the low-flow tubing used in cell wash protocols dispenses fluid so slowly that aspiration must be delayed to allow the fluid to reach the well.



Use this control to turn on the vacuum pump and begin aspiration only after the specified volume is dispensed. For cell wash assays, specify at least 10 µL/well.

For small dispense volumes, BioTek recommends setting the vacuum-on volume to equal your dispense volume. Refer also to application notes on the BioTek web site for more information (www.biotek.com).

Vacuum Filtration for Filter Plate Assays

With the optional vacuum filtration accessory kit, you can process most standard-size filter-bottom microplates.

To perform filter plate assays:

- Set up the Vacuum Filtration Accessory on page 31
- Install the Vacuum Filtration Plate Carrier on page 124
- Change the Plate Carrier Setting (Keypad) on page 125 or Change the Plate Carrier Setting (LHC) on page 124
- Create a Vacuum Filtration Protocol on the facing page
- Set up the Vacuum Filtration module
- Install Vacuum Filtration Plate Carrier
- Create a Vacuum Filtration Protocol on the facing page

Find guidelines for controlling the vacuum level in the EL406 Getting Started Guide and detailed maintenance procedures in the operator's manual.

Maintenance guidelines: Maintaining the Vacuum Filtration System on page 187

Recommendations for best performance:

Here are some guidelines to achieve the best performance of your filter plate assays:

- Shake the plate to suspend the beads before aspiration. Enable the wash cycle option to shake the plate after the dispense and before aspiration. Also consider creating a multi-step protocol that begins by shaking the plate.
- Experiment with the two parameters, aspiration time and vacuum level, to determine the best combination of settings for your assay. Start with a brief time period and low vacuum to avoid lodging the beads in the filter material.
- Maintain consistent vacuum during the process with a tight seal:
  - Use new or defect-free filter plates and make sure they are seated perfectly in the carrier;
  - Make sure all tubing is connected correctly, and leak-free.

Expect to spend some time experimenting with different pressure settings. Follow the filter plate manufacturer's recommendations, if available. Generally, bead assays prefer low pressure and DNA separation assays prefer higher pressure. The

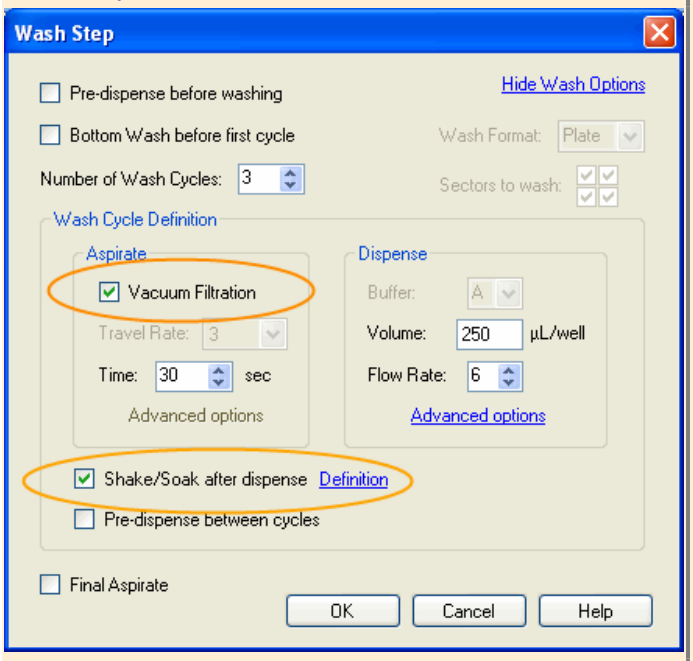
best performance in tests at BioTek were seen when the pressure was set to 2.5 inHg.

Create a Vacuum Filtration Protocol

You may want to begin the protocol with a Shake step to suspend the beads.

LHC:

1. Click (or select File>New).
2. Select the plate type.
3. Specify a Protocol Name.
4. Click W-Wash
5. Click **Show Wash Options**
6. Fill the checkbox for Vacuum Filtration and specify the vacuum duration, Time. Optionally, do the same for the Final Aspirate.



7. Fill the checkbox for Shake/Soak and specify the shake duration.

Keypad:

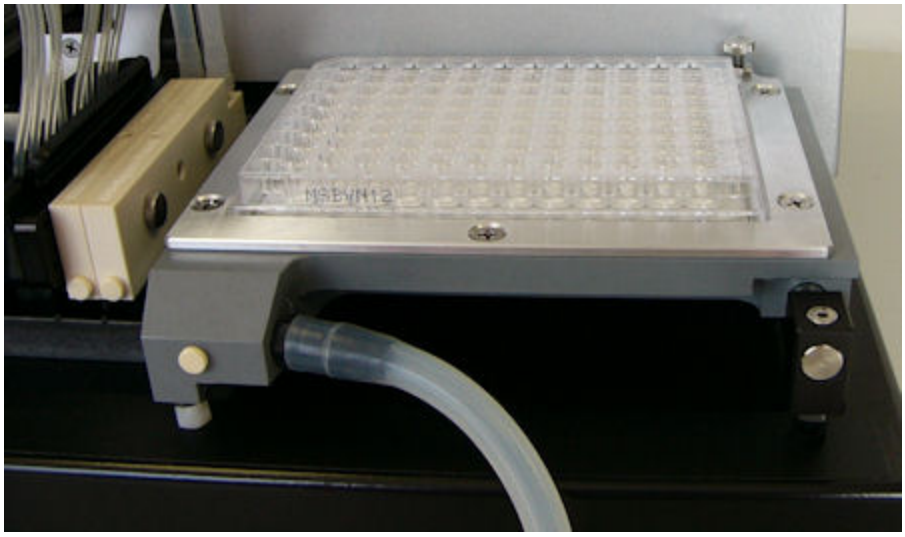
1. At the main menu, select **DEFINE>CREATE** and assign a unique name to the protocol.
2. Select the plate type.
3. Select **ADD>WASHR>WASH**
4. Select **ASPIR>VAC**
5. Specify the duration to apply vacuum.
6. Select **OPTS>MIDCYC** and add a **Shake** duration to follow each dispense to suspend the beads before applying the vacuum.
7. Select **OPTS>POST** to either disable the Final Aspirate or make sure it is defined for vacuum filtration.
8. Experiment with other parameters, like number of cycles, to optimize the protocol for your assay.

Experiment with different settings to determine the optimal parameters to meet your goals.

- Important: Be sure to change the Plate Carrier setting to match the installed carrier.

Remember to specify the type of aspiration to perform in a Final Aspirate. Keypad users: select **OPTS>POST** at the Wash Step Parameters menu.

Install the Vacuum Filtration Plate Carrier



Vacuum Filtration Plate Carrier

Connect the tubing to the pressure gauge before installing the carrier.

After connecting the tubing:

1. Look at the underside of the carrier to visualize how it fits in place: its two legs will be positioned on the left and the curved hollow fits over the transport rail on the right. Note the small slit adjacent to the curved hollow. This slit fits into the **Y-axis Carrier Arm**.
2. Position the carrier with the evacuation tubing facing forward. Aligning the curved hollow with the transport rail, place the carrier on the rail so it sits properly on the transport rail and into the slot on the **Y-axis Carrier Arm**.

- **Important**: Change the instrument's **Plate Carrier** setting to match the installed carrier before running a protocol.

Change the Plate Carrier Setting (LHC)

The instrument's onboard settings must match the installed hardware.

When you physically change the plate carrier to perform special assays, you must also change the instrument carrier setting to direct the devices to higher or lower positions to accurately address the wells:

1. Select **Tools>Instrument Utilities**.
2. On the General Settings tab, use Plate Carrier Selection drop-down list to identify the currently installed carrier:
   - STD - Standard carrier
   - VAC - Vacuum filtration carrier.
3. Click **Send**.

Change the Plate Carrier Setting (Keypad)

The instrument's onboard settings must match the installed hardware.

When you physically change the plate carrier to perform special assays, you must also change the instrument carrier setting to direct the devices to higher or lower positions to accurately address the wells:

1. Press **Setup Menu** (button in center of keypad).
2. Select → and then **CARR**.
3. Select the currently installed carrier by pressing its Soft-key:
   - STD - Standard (Magnet Ready) carrier
   - VAC - Vacuum filtration carrier.

Biomagnetic Separation - Magnetic Bead Assays

The microplate carrier supports placement of a magnet under the microplate. The magnet induces magnetic beads to settle at the bottom of the wells to help retain them during a wash protocol's aspirate cycle.

The EL406 supports standard microplates and these magnets available for purchase from BioTek:

Plate Type	Magnet	PN
96-well	96 Flat Magnet	7103016
	96 Ring Magnet	7102216
384-well	384 Flat Magnet	7103017
	384 Ring Magnet	7102215

You can use a different magnet if it fits in the carrier and accommodates your plates. Contact BioTek TAC or visit the Customer Resource Center at www.biotek.com to obtain a drawing of the carrier with its dimensions.

Persons with pacemakers/implants should avoid direct contact. Keep all magnetic media, watches, and sensitive electronic devices away from the magnets. Credit cards, tapes, and disks can be erased in the presence of a magnetic field. Bodily harm [pinching of hands and skin] can result if magnets are not handled correctly. Maintain distance between two or more magnets.

Handling and Cleaning the Magnets

For best magnet strength and bead retention, the bottom of the microplate must be as close to the magnet as possible. We recommend using flat-bottom plates with minimal support "webbing" between the sides of the outer wells and the plate skirts.

Handle the magnets with care. Avoid direct contact with the magnet material. Keep loose ferrous material away and do not attempt to disassemble.

The magnet should be stored in a cool, dry environment and should be cleaned with a damp cloth and mild detergent when exposed to harsh solvents. Do not autoclave.

To install the magnet in the proper orientation:

- Flat magnet: place in the plate carrier so the text on the side of the magnet is readable;
- Ring magnet: place in the plate carrier with the small round magnets visible, facing upwards.

Realign the BioStack with the Magnet Installed

Using the magnet increases the effective height of the carrier surface (generally by at least 7 mm). This shift in the plate position requires a comparable adjustment to the BioStack's gripper movement. Realign the BioStack before using it with the magnetic bead assays.

Refer to the BioStack Operator's Manual for detailed instructions of the alignment procedure. To help get you started:

1. Place the magnet in the carrier and a microplate on top of it.
2. Launch the **BioStack Alignment Utility**:

LHC	Keypad
Tools> BioStack Utilities> Alignment Utility	SETUP >→ BIOSTK > ALIGN

3. HOME the BioStack and Begin Realignment.
4. Lower the claw until a 0.050" (1.3 mm) gap between the bottom of the plate and the top of the gripper fingers is achieved and save the gripper position.
5. Put the microplate in the input stack and Verify the alignment.

 Remember to realign the BioStack for non-bead assays, when applicable.

Perform Magnetic Bead Assays

For the best results when performing biomagnetic separation assays:

- Use the Manifold Stop Screw Adjustment Kit, if necessary.
- Realign the BioStack with the Magnet Installed
- Change the Magnet Height Offset
- Optimize Magnetic Bead Protocols below

Optimize Magnetic Bead Protocols

Here are some suggestions to consider for optimizing your magnetic bead assays:

- **Plate Type**: Flat-bottom plates are recommended for magnetic bead assays because more of their well surface sits closer to the magnet, resulting in increased magnet strength, than with other plate formats. If you must use round-bottom plates, increase

the between-cycle soak time to improve bead separation during processing.

- **Shake/Soak Step**: Begin the protocol with a delay to let the magnetic beads settle. Also, specify a mid-cycle soak to let the beads settle after fluid is dispensed to the plate, e.g. include a 60 second soak before and between cycles.
- **6CW Aspirate Travel Rate**: select 6CW for the aspirate travel rate. The CW travel rates are designed to minimize disruption to cell layers on the bottom of the well. The same principle applies to magnetic bead assays.
- **Adjust the Aspirate height**: increase the aspirate height setting (Z-axis) which will increase the residual fluid in the wells but also preserve the beads. Good results were obtained when keeping the aspirate height around 8.0 mm above the plate carrier for all but a last Final Aspirate (disabled between cycles).
- **Adjust the Aspirate position**: When using Flat Magnets below, position the aspirate tubes near the sides of the wells (X-axis), if possible, to improve bead retention.
- As always, before running assays, we recommend testing new protocols using deionized or distilled water and a little Tween® 20 with the desired microplate and a magnet installed.

Flat Magnets

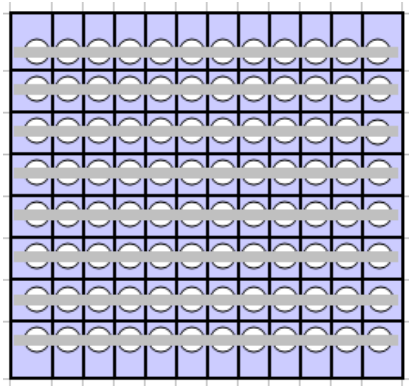
Use this information about the flat magnets to fine-tune wash protocol settings:

96F Magnet	7103016
384F Magnet	7103017

The 96- and 384-well magnets are structured differently. Their force fields traverse the magnet in opposite directions. Magnetic beads in the wells will be drawn to the center. For the best bead retention, reposition the aspirate tubes in the proper axis:

96-well Flat Magnet PN 7103016

The magnetic force (approx. 6800 Gauss) is distributed in a horizontal pattern, row-wise, across the plate. Magnetic beads are pulled to the center, across the well in flat-bottom plates and to the button in round-bottom plates. Increasing the aspirate height to increase the amount of residual in the well may improve performance.



Aspirate Settings:

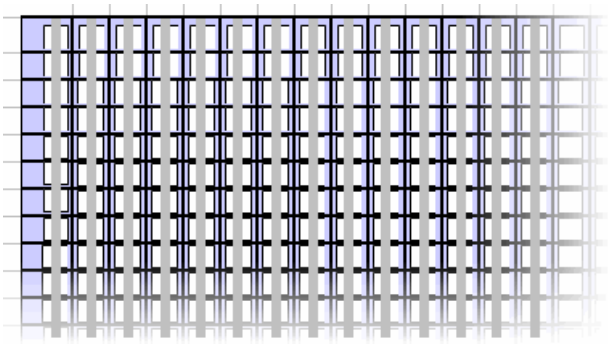
Adjust the Y Position to align the aspirate tubes near the well walls, if

available. Increase the Aspirate Height (Z axis), leaving more residual volume in the wells.

384-well Flat Magnet PN 7103017

The magnetic force (approx. 4300 Gauss) is distributed in a vertical pattern, column-wise, across the plate.

Adjust the X Position to align the aspirate tubes away from the center of the well, near the well walls.



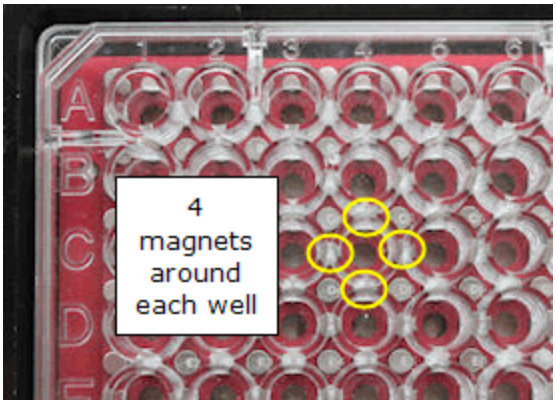
Ring Magnets

Use this information about the VP magnets to fine-tune wash protocol settings:

96 Ring Magnet	7102216
384 Ring Magnet	7102215

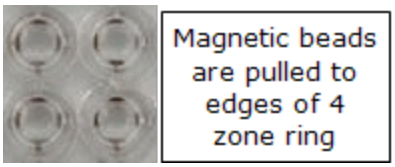
96 Ring Magnet
PN: 7102216

This magnetic bead separator uses 329 of VP's 52 MGO magnets (7094 Gauss). The magnets are arranged around each well, pulling the magnetic beads to the bottoms and edges of the wells. Aspirate from the center of the well (the default position), when using this magnet.



384 Ring Magnet
PN: 7102215

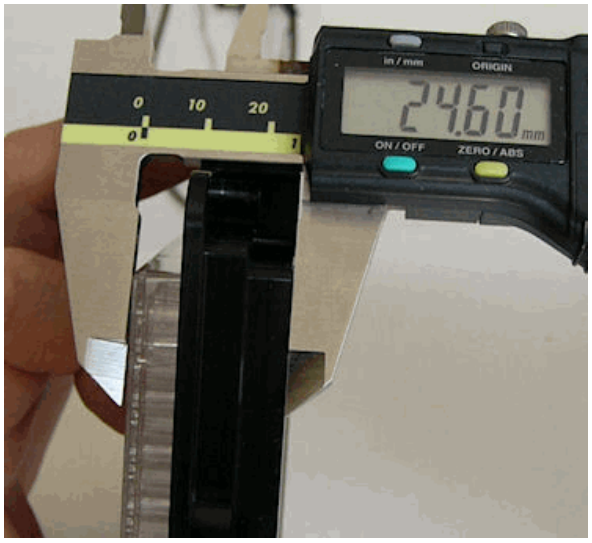
This magnetic bead separator uses 425 of VP's 52 MGO magnets (6994 Gauss). The magnets are aligned with the intersections of the wells, pulling the magnetic beads to the bottoms and edges of the wells. Every well is circled by 4 magnets.



Aspirate from the center of the well (the default position), when using this magnet.

Determine Magnet Height Offset

Use a caliper for the best results or another measuring device.



1. Measure the combined height of the plate, magnet, and adapter in millimeters.
2. Measure the plate only.
3. Calculate the offset: (plate + magnet + adapter) - plate = Magnet Adapter Height Offset

1. Put a magnet in the adapter and the type of plate you will be using on top of it.
2. Measure the distance from the bottom of the adapter to the top of the plate in millimeters (mm).
3. Remove the plate, and measure it alone.
4. Subtract the stand-alone plate measurement from the measurement value of the combined elements. This is the offset value to use.

Example:

25.6 mm = 96-well Corning microplate + 96-well flat magnet + adapter

- 14 mm = 96-well Corning microplate

11.6 mm = Magnet Height Offset

Bottom Wash

Bottom washing adds an initial dispense/aspirate sequence, another wash cycle, to the specified number of cycles. Fluid is simultaneously dispensed and aspirated to create cleaning turbulence (at the specified height). The manifold descends to aspirate again and ends with a final dispense to fill the wells.

Buffer: Select the buffer to use, if applicable.

Bottom Wash Volume: Enter the volume of wash solution to dispense per well during the bottom wash.

Flow Rate: Set the rate. Valid range is 3-11. The cell wash rates, 1 CW and 2 CW, which use low-flow tubing, when available, are not recommended. Cell wash options are designed for gentle washing, while bottom wash is designed for vigorous washing.

Advanced Options

Parameters	Description
Bottom Wash Height: Z-axis Position	The distance between the bottom of the aspirate tubes and the carrier surface. The valid range is 12 to 175. The value in mm is displayed on screen.
Horizontal X-axis Position	The left and right position of the dispense tubes when the carrier is beneath the manifold.
Horizontal Y-axis Position	The front-to-back carrier position (or Y axis) to align the microplate with the manifold tubes during a dispense. The range depends on the model.
Delay start of Vacuum	until dispensing this volume. 3000 µL max

Washer Flow Rates

Washer Dispense Parameter

The default setting for flow rates is 7, in the middle of the range for standard tubing rates 3-11, 3 is the slowest, 11 is the fastest. Slower rates are recommended for viscous fluids.

Dual 96-Tube Manifold: When this manifold is installed, you can select rate 1 or 2 to use the low-flow tubing to dispense fluid slowly and gently to avoid damaging well contents.

- 1536-well hardware uses the 128-tube aspirate manifold and the 32-tube Syringe dispenser, so these values do not apply.

	Rate	µL/tube/second	
		96 tube	192 tube
Cell Wash	**1**	116*	**NA***
Cell Wash	**2**	134*	**NA***
	3	204	102
	4	244	122
	5	273	136.5
	6	306	153
Default	**7**	325	162.5

	Rate	µL/tube/second	
	8	352	176
	9	375	187.5
	10	391	195.5
	11	418	209

*Cell Wash flow rates direct fluid through special low-flow tubing, which is not compatible with the single 96-tube and 192-tube wash manifolds.

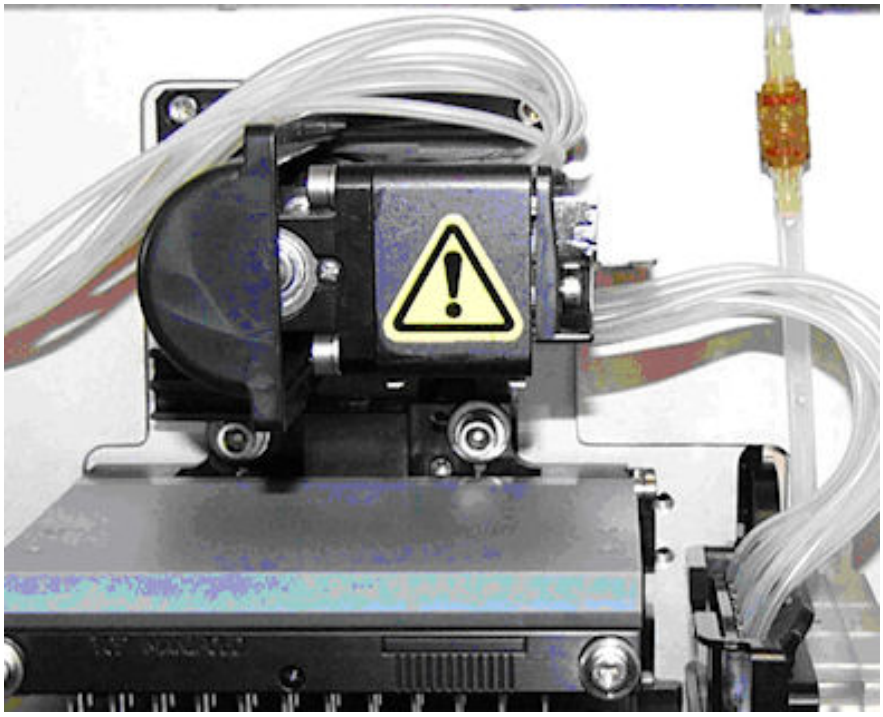
Aspirate Travel Rates

Rate	mm/sec	
1	4.1 +1	Slowest
1 CW	4.1	
2	5.0 +1	
2 CW	5.0	
3	7.3 +1	Default rate
3 CW	7.3	
4	9.4 +1	
4 CW	9.4	
5	9.4 +2	Fastest
6 CW	14.7	Recommended rate for Cell Wash protocols

CW rates are specially designed travel rates that minimize turbulence in the wells for Cell Wash protocols.

Standard rates (1-5) show two speeds as they approach the well because they slow down to 1 or 2 mm/sec before reaching the aspirate height to provide more time to aspirate the fluid, improving evacuation. Conversely, the CW rates do not change speeds, they move into and out of the well as quickly as possible to limit turbulence.

Peri-pump Peristaltic Dispenser



- Some models do not include a Peri-pump dispenser. When present it is installed above the wash manifold.

Quick Dispense-Prime-Purge

In addition to controlling the Peri-pump with defined protocol steps, the EL406 keypad offers a Quick Dispense-Prime-Purge option using the keypad. (0.5 µL dispensing is not available; you must create a protocol to dispense this volume.)

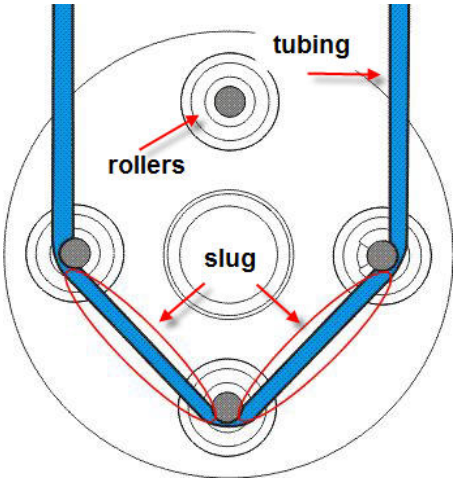
Dispense Cassettes

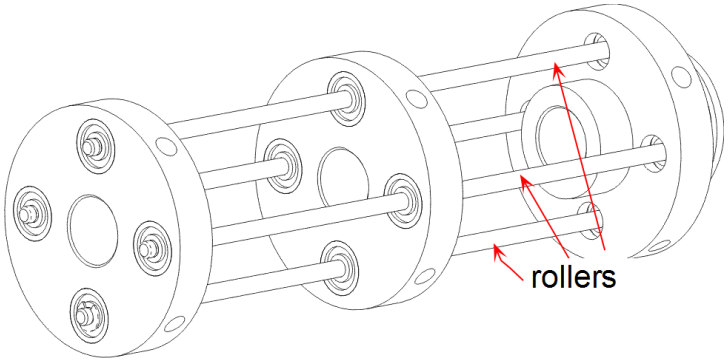
- **Important**: It is imperative that the cassette type setting onboard the instrument match the installed cassette! The cassette type is displayed in the Main Menu and Quick Dispense menu: PER-#

- Install the Dispense Cassette on page 39
- Change the cassette type setting on page 41
- Dispense Cassette Diagram on page 38
- Release the tension on the dispense cassette on page 136

Peri-pump: How it works

The Peri-pump component dispenses fluid using a peristaltic pump. It works by expelling a portion of fluid trapped between advancing rollers. The tubing is pinched by the rollers to form a full aliquot or **slug**.





Peri-pump drum

The volume of fluid in a single slug depends on the tubing size and linear distance between rollers. As the rollers spin, the fluid advances and is expelled at the tip in finite aliquots or slugs. The volume of each slug is the amount of fluid squeezed between two adjacent rollers.

The stepper motor turns the pump a determined number of steps. The pump drum can be advanced in smaller increments than the distance between rollers to expel a fraction of the full aliquot. However, the volume of the fractions is variable, depending as it does on many factors (exact rotor position, tubing tension, tip geometry, etc.). The variation between volumes of fractional aliquots is significantly higher than between dispenses done with full aliquots. Thus, **the best possible performance and reproducibility from dispense to dispense is done in full aliquot increments**. That is, specifying a 1 µL dispense volume when using the 1 µL cassette, 5 µL dispense volume when using the 5 µL cassette, and 10 µL dispense volume when using the 10 µL cassette. The exception is for instruments that support 0.5 µL dispensing. These late-model instruments apply an offset to the stepper motor to ensure precise and repeatable dispenses using a 1 µL cassette to dispense 0.5 µL aliquots, which is the only half increment permitted. Only a 0.5 µL dispense volume can be requested, not 1.5 or 2.5 µL, for example.

The tubing cassettes are calibrated by stretching the tubing to the size required to accurately dispense the expected volume per aliquot. BioTek calibrates cassettes to meet the **specifications** before shipping them. Over time the tubing's properties

will alter slightly, but the cassettes can be recalibrated to restore expected performance, in most cases.

- **See Performance Specifications on page 15**.
- **See Recalibrate the Peri-pump Dispense Cassette on page 211**.

Recommended priming volumes for the Peri-pump

Generally, the recommended prime volume is three times the dead volume, where dead volume is the total internal volume of the fluid path.

Cassette Type	Dead Volume
1 µL	1.20 mL
5 µL	4.23 mL
10 µL	7.36 mL

However, a primary advantage of the Peri-pump dispenser is its entirely visible fluid path. This allows you to prime the tubing until all visible signs of air bubbles are dissipated.

At the start of the day:

Prime the tubing to prepare for a dispense run.

1. Reload the cassette and fill the supply vessel:
   - When dispensing solutions not effected by water, prime with the dispense fluid.
   - When dispensing protein solutions, first prime the tubing with a buffered saline solution to remove any traces of water in the tubing, then, prime with the dispense fluid.
2. Hold the **Prime** button on the keypad until fluid flows into the priming trough and all visible air bubbles have been removed.

At the end of the day:

Purge the tubing to reclaim the dispense fluid, then Prime the tubing to flush it clean.

1. Hold the **Purge** button on the keypad until the tubing appears empty.
2. Replace the supply vessel with the appropriate rinse fluid:
   - When dispensing water soluble solutions, use deionized or distilled water.
   - When dispensing protein solutions, first prime the tubing with a buffered saline solution to remove protein particles, then, prime with deionized or distilled water.
3. Hold the **Prime** button on the keypad:

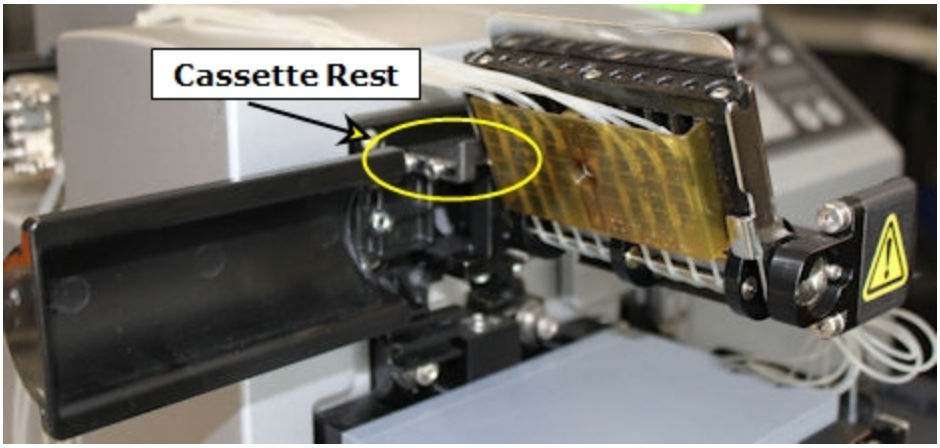
- 1 µL cassette = 5 seconds
- 5 µL cassette = 7 seconds
- 10 µL cassette = 10 seconds.

Release the tension on the dispense cassette

Important information about dispense cassettes!

When not in use, BioTek recommends releasing the tension on the cassette. This practice extends its life, preserving the tubing's integrity.

To release the tension:



1. Open the **Pump Cover**.
2. Pull out the **Cassette Rest**.
3. Release the spring-loaded latch that holds in place the **Tube Tensioner** attached to the pump's stainless steel plate. It will rest against the cassette rest.

Peri-pump Dispense Step

P-Dispense Add a Peri-pump dispense step to the protocol:

LHC	Keypad
Click **P-Dispense** and define the parameters	Select **PERIP**>**DISP** or **HLF-D***

* 0.5 µL dispensing is supported on certain late model instruments.

Define the Dispense Step

The LHC and Keypad display these parameters in distinct sequences:

Dispense Volume: Enter the per well volume in microliters. The recommended values for each cassette type are:

- 1 µL cassette = 1 - 50 µL or 0.5 µL for certain late-model instruments
- 5 µL cassette = 5 - 2500 µL
- 10 µL cassette = 10 - 3000 µL

Select the **Flow Rate**

Cassette Type	1 µL	5 µL	10 µL
	(µL/sec/tube)		
Low	50	120	140
Medium	60	140	160
High	64	160	180

When dispensing 0.5 µL with the 1 µL cassette the average flow rate values are:

1 µL Cassette	0.5 µL Dispense
	(µL/sec/tube)
Low	52
Medium	54
High	56

Cassette Type Requirement

LHC	Keypad
☑ Optionally, Require (a) specific cassette type to run this protocol. • Fill the checkbox to ensure users install the correct cassette type when running this protocol. • Leave the checkbox empty to allow any cassette type to be used.	• Select **ANY** to permit any cassette type to be used for this protocol; • Select a cassette type to engage the Cassette Requirement Mode option.

Note: Half-microliter (0.5 µL) dispensing requires a 1 µL cassette. The Cassette Requirement Mode behavior is implemented whenever a 0.5 µL volume is requested. Make sure a 1 µL cassette is installed and the Cassette Type setting matches it.
When supported, only a 0.5 µL dispense volume can be requested, not 1.5 or 2.5 µL, for example. Perform two dispense steps, one for the half microliter and another at a full increment to achieve these volumes.

Pre-Dispense

When enabled, the Peri-pump primes into the priming trough immediately before dispensing to the plate. Pre-dispense is recommended for most applications. It normalizes the tips, to correct for evaporation, for example, to ensure precise fluid distribution.

See Protocol Parameters Tables on page 73 for details about the remaining parameters.

Require a Specific Peri-pump Cassette

When defining a Peri-pump step you can require a specific cassette type. Choose the desired behavior when the required cassette is **not** installed at runtime:

- **Prompt** the user to confirm: provides the best protection against an unintentional mismatch. If the cassette type itself has been physically changed to match the protocol, but the instrument's setting has not been updated, this option changes the EL406's setting upon confirmation. However, if the cassette has not been changed to match the protocol, users are given a chance to cancel the run, fix the error, and rerun the protocol.
- Return an **Error** code: gives robotics programmers the ability to design and run unattended processing routines without fear of a message screen interrupting the operation.
- Automatically **Set** the cassette type: this option changes the EL406's setting without a confirmation. This option is for advanced users only.

  - No action is taken when the cassette type setting matches the protocol's required cassette type.

Note: The **Cassette Requirement Mode** is automatically enforced when a 0.5 µL dispense is requested. In this case, make sure a 1 µL cassette is installed.

LHC	Keypad
1. Select **Tools>Instrument Utilities>Peri-pump Dispenser**.	1. Press **Setup Menu** (button in center of keypad). 2. Select **PERI** and then → and then **MODE**.

2. Select the desired Cassette Requirement Mode behavior:
   - PROMPT
   - ERROR
   - SET

3. Select the desired mode by pressing its Soft-key:
   - PROMPT
   - ERROR
   - SET

Change the Peri-pump Dispense Pattern (Keypad)

LHC users: Change this setting in the **Instrument Utilities**.

Select the fill pattern: by column or row for processing 384- and 1536-well plates.

 Choose row for faster throughput or column for more precision.

For high-density plates, the 8-tip manifold must address the plate multiple times to fill it. Column-wise dispensing fills each column before moving to the next. Row-wise dispensing fills the first 8 rows, then reverses direction to fill the next 8 rows, and so on. Once it is defined, your pattern preference will apply to all runs, Quick Dispenses and protocols.

1. Press **Setup Menu** (button in center of keypad).
2. Select **PERI** and then **PATRN**.
3. Select the desired dispense pattern by pressing its Soft-key:
   - COL - column
   - ROW - row.

Settings for 0.5 µL Dispense

LHC: Tools>Instrument Utilities>Peri-pump Dispenser

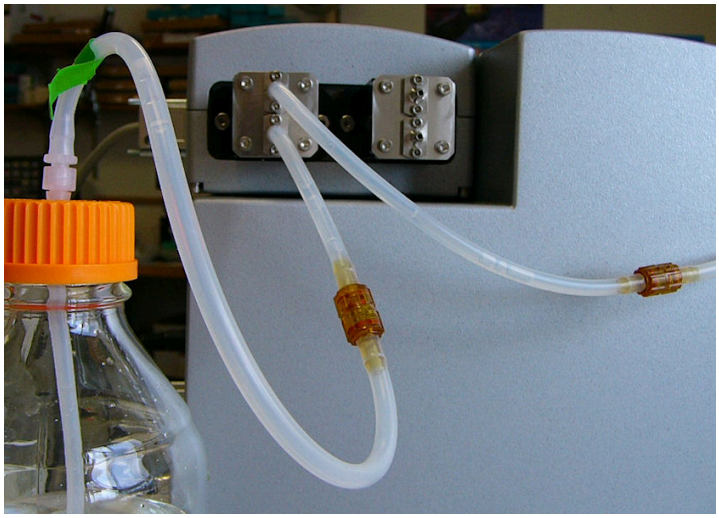
Keypad: Setup Menu>PERI>PUMP>HALF

 Do not alter these settings without precise instructions from BioTek.

Peri-pump offsets are required to ensure precise and repeatable dispense performance when dispensing 0.5 µL. The default values were determined after thorough testing and should not be changed. This control is provided in anticipation of potential future changes to the 1 µL cassette tubing. In that case, specific instructions to alter the offsets will be provided.

Contact BioTek TAC for assistance if you have questions about dispense performance at 0.5 µL.

Dual Syringe Dispenser



- Some models do not include a Syringe dispenser.

Quick Dispense

In addition to controlling the Syringe dispenser with defined protocol steps, the EL406 keypad offers a Quick Dispense menu.

How it works

The Syringe dispenser uses two positive-displacement syringe-type pumps and distinct fluid paths to accurately deliver buffer, reagents, and other fluids.

See Syringe Dispenser- Autoclavable vs. Non-autoclavable on page 147 to learn the difference between these two options.

Prime the Tubing

- How to Prime the Syringe dispenser below
- Recommended prime volumes for the Syringe dispensers on the facing page
- See also: Syringe Dispenser Maintenance on page 193

How to Prime the Syringe dispenser

Priming the tubing to remove all air bubbles is critical for accurate dispensing. There are several ways to make sure the tubing is primed:

Quick Prime using the Keypad

1. Select **QUICK** at the main menu.
2. Press **PUMP** until the Syr-A or Syr-B or Syr-BOTH menu appears, if necessary.
3. Press **PRIME** as many times as necessary. 5000 µL is dispensed each time.

Add a Prime step to the Protocol

See Syringe Prime Step on page 1.

Similarly, always include a Pre-dispense (or tip prime) in a Dispense/Wash step.

Keep the tubing wet during idle time

Turn on AutoPrime for the Syringe Dispenser on page 156

Recommended prime volumes for the Syringe dispensers

The predefined maintenance protocols for the syringe dispensers fully prime the system, pumping 40 mL through the tubing and pump:

- **S-DAY_RINSE_A&B** - to fully prime both Syringe systems
- **S-DAY_RINSE** - defined for Syringe A only

Modify these protocols as needed when changing fluids and performing other tasks. For example, make a copy of S-DAY_RINSE for Syringe B or edit the protocol to add another prime cycle when changing fluids to make sure all previously used fluid is expelled and replaced with the new liquid.

 The approximate dead volume for each Syringe dispenser system is 12 mL without Buffer Switching. The Buffer Switching unit adds 5 mL per fluid path. Generally, three times the dead volume completely primes the system. BioTek recommends modifying the predefined prime protocols when using Buffer Switching to account for the difference in fluid paths, e.g. increase the number of prime cycles. When using precious fluids, e.g., expensive reagents, you can change the prime parameters: reduce the volume or number of cycles specified in the predefined protocols or create your own protocols. Use the priming trough inserts to capture expensive reagents when priming.

Syringe Dispense Step

S-Dispense Add a Syringe pump dispense step to the protocol:

LHC	Keypad
Click **S-Dispense** and define the parameters	Select **SYRNG**>**DISP**

Define the Dispense Step

The LHC and Keypad display these parameters in distinct sequences:

Select the Syringe to dispense with:

- **A** or **B** or **Both** dispensers: without Buffer Switching module.
- **Buffer Switching options**: when this module is installed choose a compatible supply option:
  - for **Syringe A** select A1 or A2
  - for **Syringe B** select B1 or B2
  - or select one of the options for **Both** syringes

- **Both**: When both Syringe dispensers are used, they simultaneously dispense the specified volume/well to two adjacent columns in a 96-well plate, to odd numbered and then even numbered rows in a 384-well plate, and every 5th column in a 1536-well plate. Remember to create **two** prime steps, one for each dispenser, to precede the dispense step.

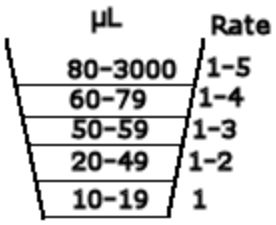
- **1536-well Flange Plates**: Use both Syringe dispensers when processing 153F plates.

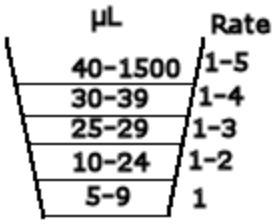
Enter the Volume to dispense per well. The valid values are 5-3000 µL depending on the plate type.

Define the **Flow Rate**: The rate at which the fluid is dispensed. Options range from 1-5, 1 is the slowest, 5 is the fastest. The valid rate is volume dependent. Lower rates are recommended for viscous fluids.

Rates are volume and plate-type dependent:

For example, rate 1 must be used when dispensing between 10-19 µL to a 96-well plate. When dispensing 20-49 µL to a 96-well plate, you can use rates 1 or 2. And, when dispensing 50-59 µL to a 96-well plate, you can use rates 1, 2, or 3. And so on, as shown in these tables.

96-well plate			16-Tube	8-Tube
	**Volume (µL)**	**Rate**	**µL/sec/well**	
	10-19	**1**	450	140
	20-49	1-**2**	600	209
	50-59	1-**3**	750	279
	60-79	1-**4**	900	350
	80-3000	1-**5**	1000	420

384-well plate			
	**Volume (µL)**	**Rate**	**µL/sec/well**
	5 -9	**1**	225
	10-24	1-**2**	300
	25-29	1-**3**	375
	30-39	1-**4**	450
	40-1500	1-**5**	500

- Note: For 16-channel syringes the µL/sec/well rate accounts for 2 tubes/well when addressing 96-well plates and one tube/well for 384-well plates.

1536-well plate			
Volume (µL) 3-3000 The 32-tube manifold flow rates do not have minimum volumes. The µL/sec/well for each type of manifold, Small Bore (SB) and Large Bore (LB), is shown:	**Rate**	**SB**	**LB**
	1	56	125
	2	58	150
	3	60	162
	4	62	174
	5	64	187
The default rate is 3.			

Enable **Pre-dispense** to normalize the tips before dispensing. Enter the volume and number of cycles.

Define the **Columns** to dispense to (aka the Plate Map)**:**

Each button represents a column.

Select the columns to dispense to using the buttons and **Set** and **Clear** links. The buttons, which represent each column, toggle on and off when clicked. Toggle them on to dispense to the column or off to skip the column.

- Each button represents a **column**. The Syringe dispenser can only skip columns, not rows.
- The number of active column buttons reflects the Plate Type selected in the main view, which should match the plate you are dispensing to.
- Learn about Processing Patterns for 384- and 1536-well plates.

Pump Delay

When dispensing highly viscous fluids, the tubing's check valves perform more slowly. Delaying the syringe pump sufficiently to allow the specified amount of fluid to pass through the check valves before being pumped into the syringe has been shown to improve dispense accuracy.
Begin by setting the delay to 500 msec. Experiment with different settings to determine the optimal value for your fluid.

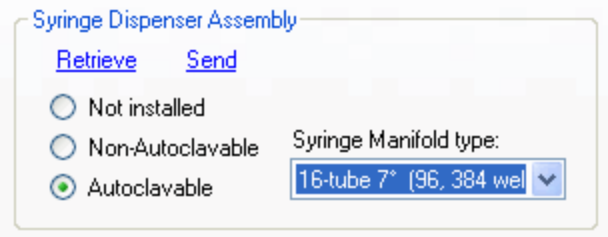
Change the Syringe Dispenser Manifold

Changing the Syringe manifold requires two steps:

- Physically changing the Syringe dispenser manifold: **See Install the Syringe Dispenser Component on page 43**.
- Updating the instrument's manifold setting; as described below.

After physically changing the manifold, perform these steps to tell the instrument which one is installed.

1. LHC: Select **Tools> Instrument Utilities> Syringe Dispenser**
   Keypad: Press the **Setup Menu** button, select **SYR** and then **MAN**.



2. LHC: Under Syringe Dispenser Assembly: Choose the option that represents the installed manifold. Look at the top of the manifold to identify its type, which is engraved on the top:

8-tube	Dual 8-tube manifolds in a single block.
16-tube	16-channel straight tubes

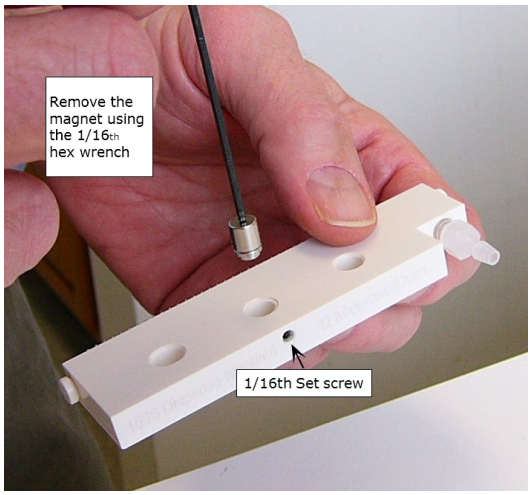
16-7 tube	16-channel angled 7°
32-tube	1536-well plates only; Model: Large Bore (LB) or Small Bore (SB)

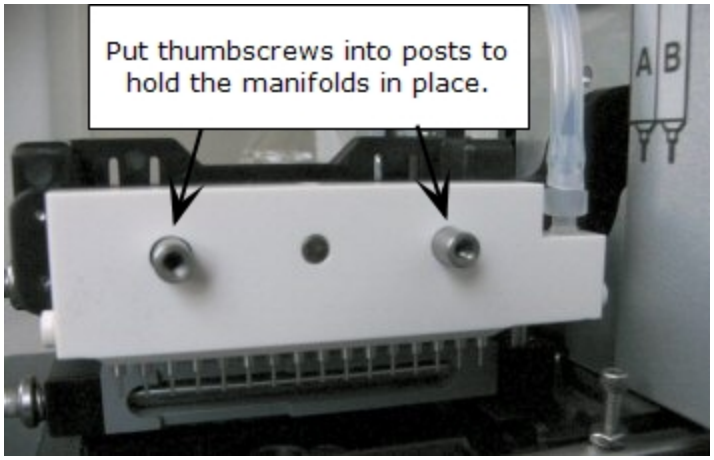
3. LHC: Click **Send** to send this setting to the instrument.

Special Procedure for Magnetic Bead Assays

Before using the dual **Syringe Dispenser** to dispense magnetic beads, you must remove the magnets that normally hold the dispense manifolds on the instrument. Two thumbscrews are shipped with the instrument to replace the magnets.

1. Use the 1/16th hex wrench (PN 48713) to loosen the set screw on top of the manifold.
2. Take advantage of the magnet's attraction to the hex wrench to remove it from the manifold.
3. Locate the thumbscrews shipped with the instrument's accessories. After sliding both manifolds onto the dispense arm, put the screws into the post to hold the manifolds in place.





- Store the magnets in the plastic pouch for potential future installation.

Syringe Dispenser Settings (Keypad)

When installing or making changes to the dual Syringe dispenser, you must update the instrument's settings to match the hardware.

Note: LHC users: select **Tools>Instrument Utilities>Syringe**

To change or review settings using the keypad:

1. Press **Setup Menu** (button in center of keypad) and select **SYR**.

Manifold (MAN)

Select MAN to define the manifold setting. Select the option that matches the dispense manifold installed:

8-tube	Dual 8-tube manifolds in a single block.
16-tube	16-channel straight tubes
16-7 tube	16-channel angled 7°
32-tube	1536-well plates only; Model: Large Bore (LB) or Small Bore (SB)

Syringe Type (TYPE)

Select TYPE to define the type of Syringe dispenser installed:

- **Non-Autoclavable (NATCLV)**: the black plastic casing of the non-autoclavable syringes are easy to distinguish from the glass and stainless steel autoclavable syringes.
- **Autoclavable (ATCLV)**: glass and stainless steel autoclavable syringes.

Calibration Data (CAL)

Select CAL to enter (or modify) the calibration data for the Syringes.

Calibration data is critical to achieving expected performance and it is unique to each individual syringe unit. BioTek prints the values on the bottom of the unit, so you have a permanent record of the values.

See Update the Instrument to use the Syringe Dispenser on page 52

Buffer Switching

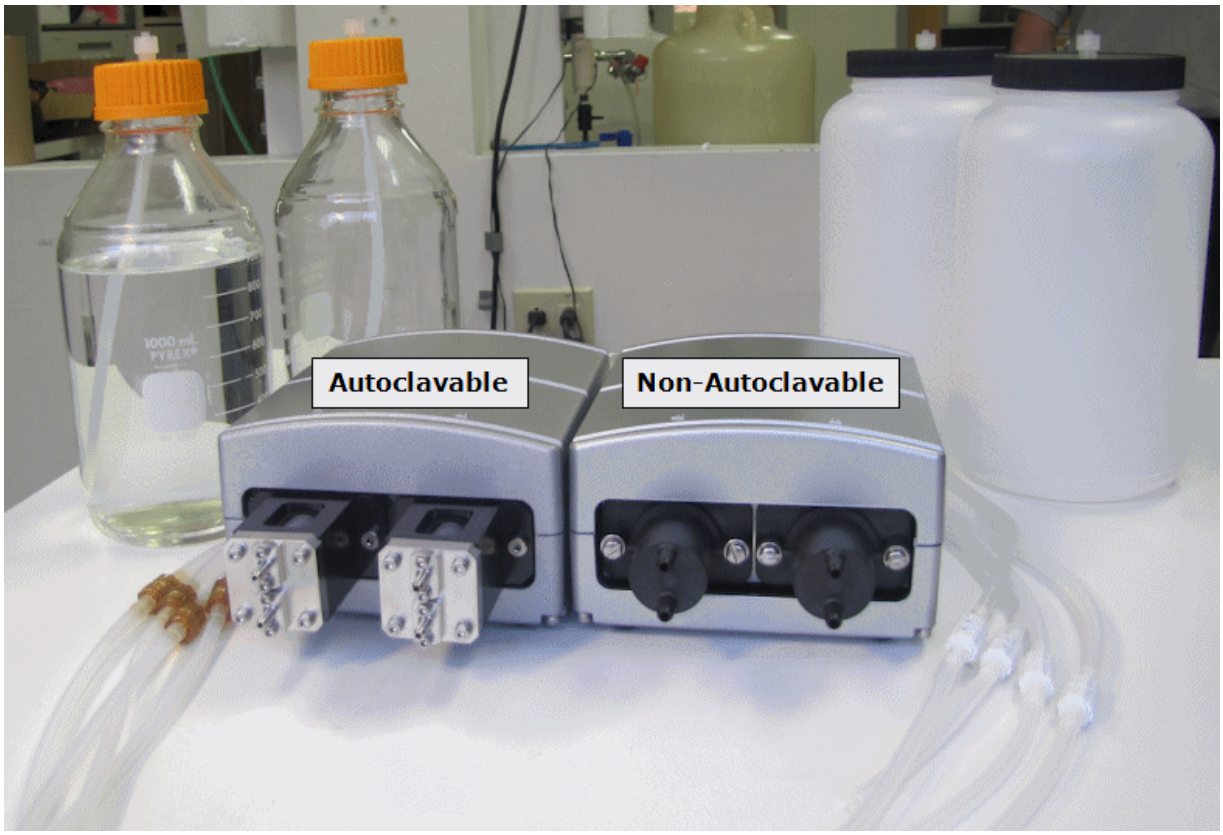
This optional accessory for the Syringe dispenser, an automated buffer switching valve box, can be installed. Make sure the instrument setting matches the current state:

- Yes = buffer switching is installed,
- No = not installed.

> **Important**: Only one buffer switching unit can be installed at a time, either the washer's buffer switching module or the Syringe's. Thus, the instrument's settings for these devices work like a toggle, when one is enabled, the other is disabled, if applicable.

Syringe Dispenser- Autoclavable vs. Non-autoclavable

Two types of Syringe dispensers are available, autoclavable and non-autoclavable. It is easy to distinguish them:



Autoclavable	Non-autoclavable
Stainless steel and glass pump heads	Black plastic pump heads
Glass supply bottles	Plastic supply bottles
Amber, transparent check valves	White, opaque check valves

EL406 Instrument Utilities: General

Plate Carrier Selection

The type of plate carrier installed on the instrument must match this setting to ensure expected performance. The instrument's basecode keeps track of the optimal positioning of its devices for each type of carrier.

- Standard (Magnet Ready)
- Vacuum Filtration: special carrier for performing vacuum aspiration or filtration assays. This setting must be selected to run a vacuum filtration protocol.

Instrument Functions (LHC Only)

- **Run the Adjust Utility**: To determine the precise positioning of the dispense and aspirate tubes.
- **Reset the instrument**: Reboots the instrument, homes all axes, and performs a system test. If you experience problems with the washer-dispenser, reset it to clear any internal errors. If problems persist, contact BioTek TAC.
- **Perform Self-check**: Homes all axes, drains the washer's and dispensers' priming troughs, and performs the system test. Any errors will be displayed.

Plate Clearance

The Plate Clearance setting adds the specified (input) value to the travel height for the selected plate type, i.e. it adds this number to the "Plate Height" value cited in the Plate Types Table. The manifold rises to this height to move from one column to the next and whenever repositioning is needed. This setting does not affect dispense and aspirate heights.

Use this setting to accommodate plates that are slightly taller than standard plates to make sure the manifolds rise high enough above the plate to prevent crashes when the plate carrier moves.

Note: Enabling the Magnet Adapter Height affects the travel height, as well as dispense and aspirate heights. The Magnet Adapter Height increases the respective values of all height parameters. Generally, it is best to keep the default setting for Plate Clearance when using the Magnet Adapter Height setting.

Default setting: 1.0 mm.

Vacuum Dissipate

Delay: 5 second default value; valid range is 0-50 seconds.

- For high-throughput plate processing, when using a BioStack and a High Flow Pump, for example, eliminate the delay: set the value to 0 seconds. For standalone use, BioTek does not recommend changing the default setting, unless you are troubleshooting.

About Vacuum Dissipation Delay: Some equipment requires a delay at the end of a run to allow air into the fluid containers to prevent the vacuum pump from overworking. A delay of 1 second/liter has proved reliable in preventing certain pumps from blowing an auxiliary 5-amp fuse (PN 46055). When necessary, set the delay to 10 seconds when using a 10-liter bottle, 20 seconds when using a 20-liter bottle, and so on.

Sensors Enabled

Fill checkboxes to enable the function; empty checkboxes to disable the function.

- **Vacuum Detection**: verifies sufficient vacuum to function properly.
- **Waste Detection**: monitors the fluid level in the waste bottles before beginning a run.

When the sensors are triggered, the LHC displays an explicit message describing the failure or required action.

- BioTek recommends keeping the detection systems activated. One exception is when running the system using air instead of fluid to dry out the components before shipping or long-term storage. In that case, deactivate the detection sensors to avoid getting errors.

Adjust Utility

LHC: Tools>Instrument Utilities>General Information

Keypad: Select UTILS>ADJST from the main menu

The **Adjust Utility** is a tool for determining the precise positioning of the dispense and aspirate tubes when addressing the plate. Generally, the components' default positions function perfectly, but certain assays may be improved by repositioning the dispense or aspirate tubes. For example, to minimize cell damage, tubes can be positioned at the sides of the wells, rather than the center. Use the utility to identify the offsets required, then, enter these offsets when defining the protocol step.

The Adjust Utility puts the plate in its run position and lets you position the plate in relation to the manifolds. You can:

- Raise or lower the manifolds in the Z-axis, to determine the optimal dispense or aspirate height, for example. The default positions are based on the plate type.
- Position the tubes in the X and Y axes by moving the plate carrier. The default position is the center of the well, 0 steps:
    - Move the carrier left or right in the X-axis. Negative offsets move left of center; positive offsets are right of center.
    - Move the carrier forward and back in the Y-axis. Negative offsets move the plate backward from center; positive offsets place the plate forward of center.

Warning: The Adjust Utility does not have limits to protect you from making bad choices. It is possible to identify dispense positions that miss the wells, for example. If incorrect values are defined in the protocol, you'll have a big mess on your hands.

Positional Limits:

The allowable ranges for positioning the devices for operation:

	X	Y	Z
Washer	-60 to 60 steps 2.74 mm offset	-40 to 40 2.96 mm offset	1 to 210 steps 26.68 mm max
Dispensers	-60 to 60 2.74 mm offset	-40 to 40 2.96 mm offset	1 to 585 steps 26.75 mm max

Run the Adjust Utility (Using the Keypad)

This page describes how to run the Adjust Utility using the keypad rather than the LHC. Find LHC instructions in the Help system.

- Learn about the **Adjust Utility on previous page.**
- Keep in mind the positional limits of the devices during operation.

1. Place a microplate on the carrier.
2. Select **UTILS** at the main menu and then select **ADJST**.
3. Select the PLATE TYPE. Only valid options for the installed hardware are presented for selection.
4. Select the device.
5. Select a run position. Only one position may be viewed at a time.
    - for the Washer choose ASPIR (aspiration) or DISP (dispense) depending on which tubes you are measuring offsets for;
    - for the Dispensers, choose FIRST column or LAST column to move the plate to that position.

6. At the AXIS screen, choose an axis, Z, X or Y. The top line of the display indicates which axis is active, and the offset position of that axis:

Axis	Washer	Dispensers	
Z-axis (up/down)	MAN	DISP	Positions the manifold or dispense arm.
X-axis (left/right)	CARX	CARX	Positions the plate carrier.
Y-axis (front/back)	CARY	CARY	Positions thee plate carrier.

7. Closely observe the position of the hardware. Use arrow keys: ◀ (reverse) and ▶ (forward) to single-step the offset in either direction.
8. When the desired offset position is found, record the position number to enter later when defining a wash, dispense, or aspirate step.
9. To quit the Adjust Utility, press Main Menu. The carrier and manifolds return to their default positions.

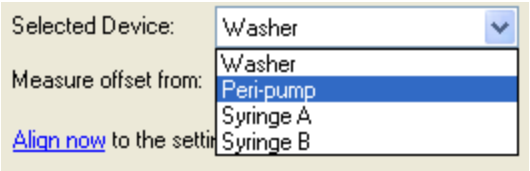
How to use the Adjust Utility- LHC

This page describes how to use the LHC to run the Adjust Utility. **See Run the Adjust Utility (Using the Keypad) on previous page**.

- Learn about the **Adjust Utility.**

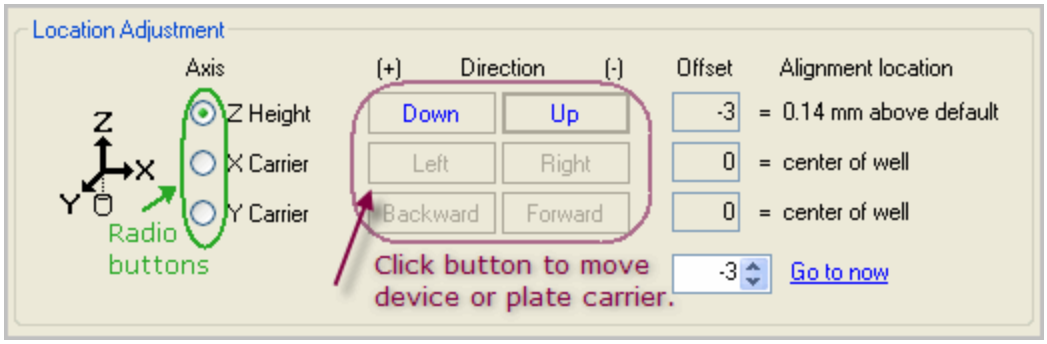
- Keep in mind the positional limits of the devices during operation.

1. Put a plate on the carrier.
2. Select **Tools>Instrument Utilities>General Settings** and click **Adjust Utility** under Instrument Functions.
3. Choose the Selected Device and the starting position to Measure offsetfrom using the drop-down lists.



4. Set the Plate Type.
5. Click the **Align now** link.

The utility positions the plate according to your selections. Take a close look at the current position of the selected device above the wells to determine the required adjustments.



6. ◉ Use the radio buttons to select the Axis you want to adjust.
7. Use the Direction buttons to move one step at a time. Press the companion button to reverse direction.

To move multiple steps at a time, use the numeric field next to the **Go to now** link. Enter a positive or negative number of steps to move the device or plate carrier and click the link.

The Offset is shown in number-of-steps and mm.

8. Repeat the process for each axis, as necessary, starting at step 6.
9. Jot down the Offset values so you can enter them when defining the protocol step.
10. Click Home All components to end the session.

AutoPrime

LHC: Tools>Instrument Utilities> AutoPrime

Keypad: Select UTIL at the main menu, then AUTPRM

Recommended for optimum performance, AutoPrime keeps the tubing wet in between runs and can be an essential part of your daily maintenance routine.

About AutoPrime

AutoPrime automatically primes the tubing whenever the instrument is idle for a specified time. Keeping the tubes wet prevents clogging and mitigates fluid evaporation at the tips. AutoPrime's submerge feature lets you soak the tubes for extended periods, which is an effective maintenance option.

The downtime interval is defined for all components, but each device supports distinct priming parameters. Devices are primed consecutively, beginning with the syringe dispensers, not concurrently.

Important: Remove the Peri-pump cassette when the Syringe dispenser is set to "Submerge."

"Submerge" is not offered for the Peri-pump because it is not a good practice for the dispense cassettes. However, because the cassette Tip Holder resides on the same dispense arm as the Syringe dispenser manifold, it will be moved into the submerge position when the Syringe tips are submerged. Either remove the cassette altogether when soaking the Syringe tips, or remove the prime trough inserts for the Peri-pump to prevent cross contamination of fluids or unintended wicking of fluid into the cassette.

- Keep in mind that any interaction with the EL406 will reset the interval clock. And, AutoPrime only runs when the main menu, quick menu, or run completion message is displayed.

- When AutoPrime is running you can press **STOP** on the keypad to stop it. It will run again the next time the downtime interval occurs.

Specify the Interval and AutoPrime Parameters

AutoPrime runs when the instrument has been idle for a specified interval. One interval setting is defined for all devices.

To set the **AutoPrime Interval**:

LHC:	Keypad:
1. Select **Tools>Instrument Utilities> AutoPrime** tab. 2. Specify the idle-time interval that will trigger an AutoPrime; up to 24 hours. 3. Enable and define the parameters for each device. Remember to set a Submerge Duration to employ this option. 4. Click **Send** to transfer the settings to the instrument.	1. Select **UTILS** at the main menu and select **AUTPRM**. 2. Specify the idle-time interval that will trigger an AutoPrime; up to 24 hours in minutes and press Enter. 3. Set the Submerge Duration, if desired. You will select the device to submerge in a subsequent step. 4. At the AutoPrime Device Menu, select the device you want to enable. 5. "**Enable AutoPrime**." 6. For each device, define the AutoPrime parameters: rate, volume, and buffer valve, if applicable. Press Enter at each screen to advance to the next.

Turn on AutoPrime for the Washer

- Learn about **AutoPrime**, if you haven't already done so.

▪ **Keypad users**: Select **UTIL** at the main menu, then **AUTPRM** and specify parameters.

1. Select **Tools>Instrument Utilities>AutoPrime**.
2. ☑ Fill the **Enabled** checkbox for the washer.
3. Set the AutoPrime Interval: enter the downtime interval that will trigger an AutoPrime.
4. Select the **Buffer** to use if you have Buffer Switching installed.
5. Set the **Volume** and **Rate.** The default values are 40 mL at flow rate 9.

6. You can **Submerge** the manifold tubes in the fluid **after** the **prime**, if desired. Set **Duration** in Hours and Minutes.

   After the dispense tubes have been primed, the manifold moves down into the priming trough that is filled with the dispensed solution. The vacuum pump is turned off and the tubes are allowed to soak. After the specified duration, the vacuum pump is turned on and the trough is aspirated.

   It takes approximately 93 mL of fluid to fill the priming trough. Be sure to specify a volume of fluid that will cover the tubes.

7. Click **Send** to transfer the settings to the instrument.

Keep in mind:

- AutoPrime can be stopped when it is underway: press STOP on the keypad.
- Any interaction with the instrument via the keypad or the LHC resets the interval clock.
- AutoPrime only runs when the main menu, quick menu, or run completion message is displayed.

Turn on AutoPrime for the Peri-pump Dispenser

LHC	Keypad
1. Select **Tools>Instrument Utilities>AutoPrime**; 2. Set the **AutoPrime Interval**: enter the downtime interval that will trigger an AutoPrime. 3. ☑ Fill the **Enabled** checkbox for the dispenser. 4. Set the **Volume** and **Rate**. The default settings are 50 µL/tube at the High flow rate. 5. Click **Send**. Wait for a confirmation message.	1. Select **UTIL>AUTPRM**. 2. Set the **AutoPrime Interval**: enter the downtime interval that will trigger an AutoPrime. 3. Select the dispenser. 4. Set the **Volume** and **Rate**. The default settings are 50 µL/tube at the High flow rate. 5. Press **Main Menu**, and look for AP in the display to confirm that AutoPrime is enabled.

Details about Peri-pump AutoPrime

Important: Remove the Peri-pump cassette when the Syringe dispenser is set to "Submerge."

"Submerge" is not offered for the Peri-pump because it is not a good practice for the dispense cassettes. However, because the cassette Tip Holder resides on the same dispense arm as the Syringe dispenser manifold, it will be moved into the

submerge position when the Syringe tips are submerged. Either remove the cassette altogether when soaking the Syringe tips, or remove the prime trough inserts for the Peri-pump to prevent cross contamination of fluids or unintended wicking of fluid into the cassette.

- Keep in mind that any interaction with the EL406 will reset the interval clock. And, AutoPrime only runs when the main menu, quick menu, or run completion message is displayed.

- When AutoPrime is running you can press **STOP** on the keypad to stop it. It will run again the next time the downtime interval occurs.

When the Peri-pump AutoPrime is Stopped

Because purging and priming the tubing is the best way to remove air bubbles and prepare the tubing for accurate dispensing, this routine is executed whenever AutoPrime for the Peri-pump is stopped:

1. **Purge**: fluid is purged from the tubing, i.e. returned to the supply vessel. The volume depends on the cassette type.
2. **Prime**: the tubing is primed with the optimal volume of fluid:

Cassette Type	Purge Volume	Prime Volume
10 µL	250	750
5 µL	150	450
1 µL	50	200

Turn on AutoPrime for the Syringe Dispenser

LHC	Keypad
1. Select **Tools>Instrument Utilities>AutoPrime**; 2. Set the **AutoPrime Interval**: enter the downtime interval that will trigger an AutoPrime. 3. ☑ Fill the **Enabled** checkbox for the dispenser. 4. Set the **Volume** and **Rate**. The default settings are 400 µL at rate 3.	1. Select **UTIL>AUTPRM**. 2. Set the **AutoPrime Interval**: enter the downtime interval that will trigger an AutoPrime. 3. Select the dispenser. 4. Set the **Volume** and **Rate**. The default settings are 400 µL at rate 3. 5. Press **Main Menu**, and look for

LHC	Keypad
5. Click **Send**. Wait for a confirmation message.	AP in the display to confirm that AutoPrime is enabled.

Note: The **Volume** dispensed is actually double the input value. The Syringe dispenser always performs two prime cycles during AutoPrime, doubling the volume. For example, when 400 µL is entered, the dispenser actually primes the tubing with 800 µL.

You can Submerge the dispense tubes in the fluid after the prime, if desired. This requires filling the **priming trough inserts**, one for each syringe. Each reservoir holds approximately 6.5 mL.

After the dispense tubes have been primed, the manifold moves down into the priming trough inserts. When both Syringes are enabled, the soak duration begins after both dispensers are primed.

The vacuum pump is turned off and the tubes are allowed to soak.

- **Important**: When using the submerge option, specify a volume that fills the priming trough inserts with sufficient fluid to cover the tubes, e.g. 6000 µL. And, be sure to use the priming trough inserts when the option is enabled. Learn more about soaking the dispense tubes...

Set the Submerge Duration and fill the Submerge checkbox to enable this option.

- Remove the Peri-pump cassette or its priming trough insert when soaking the Syringe dispensers.

Important: Remove the Peri-pump cassette when the Syringe dispenser is set to "Submerge."

"Submerge" is not offered for the Peri-pump because it is not a good practice for the dispense cassettes. However, because the cassette Tip Holder resides on the same dispense arm as the Syringe dispenser manifold, it will be moved into the submerge position when the Syringe tips are submerged. Either remove the cassette altogether when soaking the Syringe tips, or remove the prime trough inserts for the Peri-pump to prevent cross contamination of fluids or unintended wicking of fluid into the cassette.

- Keep in mind that any interaction with the EL406 will reset the interval clock. And, AutoPrime only runs when the main menu, quick menu, or run completion message is displayed.

- When AutoPrime is running you can press **STOP** on the keypad to stop it. It will run again the next time the downtime interval occurs.

Delay After Dispense

About Vacuum Dissipation Delay or Delay after Dispense:

Standard vacuum pumps (not high flow) require a delay at the end of a run to allow air into the fluid containers to prevent the vacuum pump from overworking. A delay of 1 second/liter has proven to be reliable in preventing certain pumps from blowing an auxiliary 5-amp fuse (PN 46055). When necessary, specify a delay of 10 seconds when using a 10-liter bottle, 20 seconds when using a 20-liter bottle, and so on.

By default the EL406 is programmed with a 5 second delay after every dispense; valid range is 0-50 seconds.

To adjust the delay:

1. LHC: Select **Tools>Instrument Utilities>General Settings**
   Keypad: Press **Setup Menu** and select —> twice, select **VACDIS** .
2. Set the Delay to the desired time period.
3. LHC: Click **Send**.
   Keypad: Press **Enter**.

Download Basecode (LHC Only)

Tools> Instrument Utilities>Software> Download Basecode

Keypad users: Contact BioTek TAC for guidance.

Use this feature when it is necessary to upgrade the instrument's onboard software or basecode. BioTek provides the basecode (EL406.bin) file on the **Customer Resource Center** website.

Important prerequisite: Download the basecode from our website to perform the basecode upgrade. Alternatively, you can request a CD of the basecode software.

To download basecode to the instrument:

1. Put the instrument in download mode:
2. Turn off the EL406.
3. Press and hold the **Shift** key while restarting the EL406, i.e. turn it on.

The keypad display will appear blank. If the main menu appears, start again.

4. Using the LHC, select **Tools> Instrument Utilities**, select the Software tab and click **Download Basecode**
5. Click **Browse** to the **EL406.bin** file.
6. Click **Start Download**.

- **Technical Note**: Only one of the two communication ports (COM port) on the instrument can be used at a time. They cannot be used simultaneously. You can use either the USB or the RS232 serial port to connect the EL406 to a computer or to a BioStack or similar robotic device. But you cannot use both ports simultaneously.

Upload-Download Protocols (LHC Only)

The LHC lets you transfer protocols from your computer to your instrument and back again.

Limitation: Protocols must contain only instrument-supported action steps to qualify for download. That is, the protocol cannot contain any of the LHC provided steps like Delay and Loop (buttons in the left column of the Add Step box in the main view). And, a Protocol Name is required.

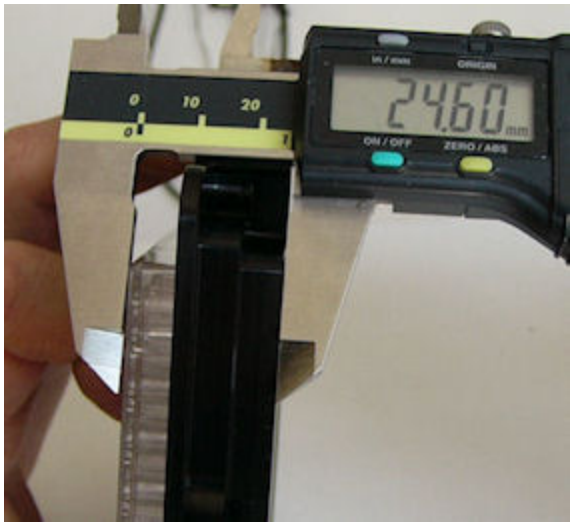
> ▪ The instrument's main menu must be displayed for the LHC to communicate with it.

1. Select **Tools>Transfer Protocols**.
2. Make sure the desired protocols are displayed: check the **Protocol Folder** path for This computer. Refresh the list of protocols onboard the Instrument by clicking the **Settings** link.
3. Highlight one or more protocols in a display box. (Hold the Ctrl or Shift key to simultaneously select multiple files.)
4. Optionally, at the top left corner of the screen, choose to Disable Editing of transferred protocols to lock the protocols from editing or deleting when they are onboard the instrument.
5. Click the applicable **Upload** or **Download** button.

The LHC will confirm the transfer or prompt you for more information. When the transfer is complete, you can manipulate the files as you normally would in their new location.

How to determine the Magnet Adapter Height Offset

Use a caliper for the best results or another measuring device.



1. Put a magnet in the adapter, and the type of plate you will be using in place on top of it.
2. Measure the distance from the bottom of the adapter to the top of the plate in millimeters (mm).
3. Remove the plate, and measure it alone.
4. Subtract the stand-alone plate measurement from the measurement value of the combined elements. This is the value to use.

- Be sure to click **Send** after inputting the offset value to update the EL406 with this Magnet Adapter Height setting.

Examples

In our testing we found these offset values to be effective:

Plate Type	With	PN	Offset Value (mm)
96 Corning	Magnet Adapter	7180011 (kit)	8.73
	Dexter 96 Magnet	7103016	10.20
	VP 96 Ring Magnet	7102216	10.48
	VP 96 Magnet	7102217	10.18
384 Corning	Magnet Adapter	7180011 (kit)	8.73
	Dexter 384 Magnet	7103017	11.20
	VP 384 Ring Magnet	7102215	11.50

Change the Washer Buffer Switching Setting

The instrument's onboard settings must match the installed hardware.

Instrument models that specify Buffer Switching ("B" is included in their model name) are configured at the factory to use the valve module to supply fluid. If you choose not to use the module and instead connect a supply bottle directly to the instrument, change the washer's buffer switching setting:

Keypad	LHC
1. Press **Setup Menu** (button in center of keypad). 2. Select **WASH** and then **BUFMOD**. 3. Select Yes when the buffer switching module is installed, otherwise, select No.	1. Select **Tools>Instrument Utilities>Washer**. 2. Change the setting for the Buffer Switching Module.

Chapter 4

Maintenance

Properly maintaining the EL406 is the key to reliable performance.

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Overview

A **Preventive Maintenance (PM)** regimen for the EL406 includes rinsing and soaking the fluid path and cleaning and/or autoclaving the various components. The level of maintenance required to keep the instrument performing as expected is dependent on several factors, including the type of fluid dispensed, the frequency of use, and the work habits employed.

The Recommended Maintenance Schedule on page 167 summarizes BioTek's recommended maintenance tasks, and indicates approximately how often each task should be performed. Daily and periodic routines and minimal guidelines for frequency are listed. Beyond that, it is difficult for BioTek to recommend a fixed frequency for each task to be performed. The frequency of conducting these tasks must be based on the risk and performance factors of your assays.

Develop a maintenance schedule for your EL406 based on the characteristics of the fluids used and the activity level. Here are some guidelines for each component:

Washer

- When using fluids prone to dry and harden quickly, the washer's dispense and aspirate tubes can clog quickly, and must be rinsed frequently and cleaned regularly. Run **AutoClean** ultrasonic cleaning regularly, when available. Otherwise, Remove and clean the washer manifold on page 185.
- If the washer will be idle for several hours or days at a time, soak the tubes to keep them in a "wetted" state. Enable **AutoPrime** if the washer is idle for more than 3 hours.
- Wash solutions affect the rinse frequency. If the solution does **not** contain surfactant, consider rinsing (or running **AutoPrime**) at least once an hour.

Peri-pump

- Purge the fluid at the end of a dispense run and flush the tubing with water (or buffered saline and then water). This is a good practice whenever the dispenser will be idle for more than an hour, and at the end of the day.
- When using the 1 µL cassette, filter fluids to 50 microns to reduce the chance of the tips clogging.
- When dispensing fluids that can crystallize or harden after use, increase the frequency of maintenance activities. Autoclave the cassette as needed.
- Keep track of the number of plates processed with a cassette to determine when the cassette has reached its expected lifetime and is due for replacement or recalibration.

Syringe Dispenser

- Perform the daily maintenance routines. Flush the dispenser with an appropriate reagent at the beginning of the day (e.g., deionized water in the morning) and at the end of a run.
- When dispensing fluids that can crystallize or harden after use, increase the frequency of maintenance activities. This is especially important for the 32-tube small bore (SB) dispensers.
- Autoclave the Syringe heads and pistons, when applicable, and the 16-tube manifolds as needed. **See Clean the Dispense Tubes on page 195**.

Recommended Maintenance Schedule

The schedule recommends preventive maintenance tasks, the frequency with which each task should be performed, and the predefined onboard Maintenance program that should be run (if applicable). **See Recommended Maintenance Schedule on the facing page**.

- It is important to note that the risk and performance factors associated with your assays may require that some or all of the procedures be performed more frequently than suggested in this schedule.

- **Water**: Daily maintenance is the key to keeping the liquid handler performing to specifications. In the maintenance procedures provided in this manual, the requirement to use distilled (dH2O) or deionized (DI) water can be met by numerous water purification methods, including MilliQ™. A minimum water purity of 2mOhm is expected.

Recommended Maintenance Schedule

Tasks	Frequency				
	Daily	Overnight/ Multi-Day	Weekly	Periodic/ Monthly	Before storage/ shipment
Washer					
Run W-DAY_RINSE	✓	✓			
Run AutoPrime	✓				
Run W-OVERNIGHT_LOOP		✓			
Run W-RINSE_AND_SOAK		✓			
Run AutoClean				✓	✓
Peri-pump					
Flush dispense cassette	✓	✓			
Record number of plates processed with cassette	✓				
Syringe Dispenser					
Run S-DAY_RINSE_A&B	✓	✓			
Run AutoPrime	✓				
Components					
Remove protein residuals and fungi growth, (if necessary)	✓		✓	✓	
Check/empty waste bottles	✓				✓
Clean bottles				✓	✓
Clean plate carrier system			✓		✓
Clean washer manifold				✓	✓
Clean aspirate and dispense tubes				✓	✓
Clean exterior surfaces and mist shield			✓		
Clean fluid inlet filter				✓	✓

Tasks	Frequency				
	Daily	Overnight/ Multi-Day	Weekly	Periodic/ Monthly	Before storage/ shipment
Clean Syringe dispenser manifold				✓	✓
Clean Syringe dispenser tubes				✓	✓
Autoclave Syringe pumps				✓	
Clean vacuum filtration system				✓	✓
Decontaminate					
Decontaminate external surfaces				✓	✓
Run W-DECONTAMINATE				✓	✓
Run S-DECONTAMINATE				✓	✓
Decontaminate vacuum filtration system				✓	
Prepare for Storage or Shipment					
Run W-LONG_SHUTDOWN					✓
Run S-LONG_SHUTDOWN					✓
Replace/Repair Components					
Replace washer manifold O-rings and channel-end seals	Annually				
Recalibrate Peri-pump cassette Find the PDF folder on the operator's manual CD for detailed instructions for two methods of recalibrating the cassette.	As Needed				
Replace Peri-pump dispense tips	As Needed				
Replace Peri-pump cassette tubing	As Needed				
Replace Syringe manifold check valves and plugs	As Needed				

Daily Maintenance

Daily maintenance involves flushing the washer and dispensers with an appropriate reagent or deionized water throughout the day. Routine rinsing helps to prevent the aspirate and dispense tubes from clogging between runs. Flushing the devices with deionized water is recommended at the end of the day for most applications.

The recommended **rinsing frequency** depends on the solutions currently in use:

- When a solution containing surfactant is used throughout the day, perform the rinsing procedure when the device is idle for more than 3 hours.
- When the solution does **not** contain surfactant, consider rinsing at least once an hour.

> ▪ The type of hardware also affects rinsing frequency. The 32-tube dispense manifolds require more diligence to keep them clog-free.

Run these protocols and enable **AutoPrime** to satisfy the daily maintenance requirements:

- W-DAY_RINSE
- S-DAY_RINSE_A&B
- P-#UL_CASS_RINSE (# represents the cassette type)

Make sure the supply bottles contain sufficient rinse solution and that the waste bottles are empty before running the protocols.

 Also see the additional maintenance procedures required when dispensing protein solutions: Removing Protein Residuals and Fungi Growth on page 175.

AutoPrime

AutoPrime automatically conditions the dispense tubes, priming them with the specified volume, after a user-specified amount of idle time. **See AutoPrime on page 153**.

- Press the **STOP** button to interrupt the AutoPrime routine when it is underway.
- Any interaction with the instrument via the keypad or the LHC resets the interval clock.

- AutoPrime only runs when the main menu, quick menu, or run completion message is displayed on the keypad.

Overnight/Multi-Day Maintenance

Overnight/multi-day maintenance involves flushing all solutions out of the instrument, and then periodically rinsing and soaking the tubes to keep them moist. Here are three recommendations for accomplishing the task. Employ the method that best suits your work flow:

Maintaining 1536-well Hardware

At the end of the day run W-DAY_RINSE to flush the 128-tube aspirate manifold. The aspirate tubes cannot completely empty the priming trough when the run is finished. Remove the fluid manually with a pipette or paper towel.

The 32-tube-SB dispense manifolds can become easily clogged. It is especially important to perform one of the overnight maintenance procedures with the suggested soaking periods to ensure trouble free startup at the beginning of the next day.

Overnight Loop

To keep the wash manifolds in a wetted condition, you can run these predefined protocols to soak the tubes for several hours at a time:

- W-OVERNIGHT_LOOP: requires the washer to remain turned on.

- W-RINSE_AND_SOAK: alternatively, run this protocol and turn off the instrument after the soak begins. The tubes will soak in the priming trough until the instrument is turned on again.

AutoPrime for Overnight-Weekend Maintenance

AutoPrime can be used to keep the manifold tubes wetted during idle periods throughout the day and then modified to soak the tubing for longer periods overnight and on the weekends. **See AutoPrime on page 153** for setup instructions.

AutoPrime Parameters	Washer	Syringe
AutoPrime Interval	15 minutes*	15 minutes*
Device	Washer	Syringe A and B

AutoPrime	Yes/Enabled	Yes/Enabled
Volume	90 mL	1000 µL
Rate	09	3
Buffer	Any	NA
Submerge Duration	120 minutes/2 hours	180 minutes/3 hours

*To repeatedly soak both devices, make sure the AutoPrime interval is less than the total combined submerge duration.

- When the EL406is set up for 1536-well washing, you can disable AutoPrime for the washer; typically the 128-tube aspirate manifold does not require the same amount of soaking as dispense manifolds.

Overnight/Multi-day Procedure: After modifying the AutoPrime parameters to submerge the tubes for several hours:

1. Put the priming-trough-inserts into the priming trough.
2. Use Quick Dispense to fill both inserts (prime cups): **DISP>PRIME** (for both Syringe A and B). Run the Prime two or three times to fill the cups.
3. When the priming troughs are filled, press Main Menu.

Submerge and Shutdown

An overnight/multi-day maintenance option for soaking the tips and turning off the instrument for overnight and weekend maintenance.

You can soak the EL406 dispense manifolds by filling the priming troughs and turning off the instrument after the soak begins. The tubes will soak in the priming troughs until the instrument is turned on again.

1. First, run **S-DAY_RINSE_A&B** but do not empty the priming trough inserts.
2. Run **W-RINSE_AND_SOAK**.
3. When the wash manifold is submerged, turn off the instrument.

The syringe dispenser manifolds will lower into their troughs.

> ▪ Remove either the cassette or the Peri-pump priming trough from the instrument before performing this procedure.

32-tube Syringe Dispenser

When using the 32-tube dispense manifold, especially the small bore (SB) model, BioTek recommends using the following AutoPrime parameters to keep the

manifold clog-free:

Volume:	1000 µL
Flow rate:	3
Submerge duration:	3 hours (requires priming trough inserts)

Overnight/Multi-day practice: After modifying the AutoPrime parameters to submerge the tubes for several hours:

1. Put the priming-trough-inserts into the priming trough.
2. Use **Quick Dispense** to fill both inserts (prime cups): **QUICK>PRIME** (for both Syringe A and B). Run the **Prime** two or three times to fill the cups.
3. When the priming troughs are filled, press **Main Menu**.

Soak the manifold tubes in cleaning fluid

Keeping the dispense and aspirate tubes in a wetted condition is required to keep them clog free. Soak the tubing in deionized or distilled water or a cleaning fluid whenever the device is not being used to ensure trouble-free performance.

Prime Step

1. Fill a supply bottle with a cleaning agent.
2. Click **W-Prime** or **S-Prime** and define parameters to fully prime the tubing, for example:

Washer	Syringe Dispenser
Volume = 500 mL	Volume = 8000 µL
Flow Rate = 7	Flow Rate = 5
Prime Low Flow = 100 mL	Cycles = 5
☑ **Submerge tips** in fluid after prime: enable option and set Duration, up to 24 hours.	

- For the Syringe manifolds: Use the priming trough inserts to submerge the tips.

- **Overnight Maintenance**: You can turn off the instrument after the tips are submerged to leave them soaking in the priming trough for an extended duration.

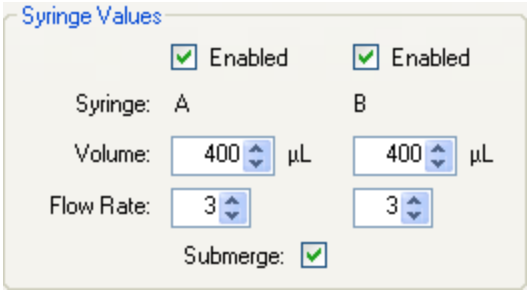
3. Click **Run**. Save the program with a memorable name for future use.

AutoPrime

Enable **AutoPrime** to ensure the tubing is soaked during downtime intervals.

It is especially important when using the 32-tube SB dispense manifolds to keep the tubes wet to prevent clogs. One option is to modify the AutoPrime parameters used for idle periods during a regular work day to soak the tubing for longer periods overnight and on the weekends.

- Be sure to use the priming trough inserts when the submerge feature is enabled. And, remove either the Peri-pump cassette or its priming trough insert.



Define the AutoPrime values to fill the priming-trough-inserts, e.g. 6000 µL, and then submerge the tubes for several hours. When AutoPrime is enabled for both dispense manifolds, the soak duration begins after both manifolds are primed, i.e. both priming-trough-inserts have been filled.

When the EL406 is set up for 1536-well washing, you can disable AutoPrime for the washer; the 128-tube aspirate manifold does not require the same level of soaking as dispense manifolds.

More about soaking the dispense tubes...

You can soak the Syringe dispenser's two manifolds, A and B, simultaneously or separately depending on how you define the prime routine. Two priming trough inserts are supplied, one for each manifold. The troughs can be used to preserve precious fluids and to soak the manifold tubes.

- Be sure to use the **prime trough inserts** to submerge the tips in fluid.

Create a Prime protocol

As an alternative to AutoPrime, BioTek recommends creating a protocol with two prime steps to soak the Syringe dispenser's tubes:

- Do not enable the submerge feature for Syringe A. Specify a volume that fills the prime trough insert, e.g. 6000 µL.
- Create a second prime step for Syringe B, with the same volume, and the submerge duration defined. Both Syringe A and Syringe B dispense tubes will be submerged in their respective prime trough inserts.

Important: Remove the Peri-pump cassette when the Syringe dispenser is set to "Submerge."

"Submerge" is not offered for the Peri-pump because it is not a good practice for the dispense cassettes. However, because the cassette Tip Holder resides on the same dispense arm as the Syringe dispenser manifold, it will be moved into the submerge position when the Syringe tips are submerged. Either remove the cassette altogether when soaking the Syringe tips, or remove the prime trough inserts for the Peri-pump to prevent cross contamination of fluids or unintended wicking of fluid into the cassette.

- Keep in mind that any interaction with the EL406 will reset the interval clock. And, AutoPrime only runs when the main menu, quick menu, or run completion message is displayed.

- When AutoPrime is running you can press **STOP** on the keypad to stop it. It will run again the next time the downtime interval occurs.

Removing Protein Residuals and Fungi Growth

Important! Solutions containing proteins, such as bovine serum albumin (BSA), will compromise the EL406's performance over time unless a strict maintenance regime is adhered to. Do not use isopropyl alcohol to flush out BSA.

When using protein solutions or similar fluids, BioTek recommends performing the following additional Maintenance procedures to thoroughly flush out protein particles and other contaminants from the fluid path.

Also note, some components can be autoclaved to sterilize them.

- **Four-liter** volumes specified in the following are approximate amounts.

- **S-DECONTAMINATE** (S-DECON): this predefined protocol specifies Syringe A. Make a copy of the protocol and modify it for Syringe B.

Daily Practice with buffer or deionized water:

If the EL406 will be idle between plates for longer than 45 minutes, flush the proteins:

1. Fill a supply bottle with deionized water. Connect the bottle to the washer or dispenser. (Buffer valve "A" for Buffer Switching models)
2. Run the applicable DAY_RINSE protocol.
3. Enable AutoPrime for 60-minute intervals.

At the end of the day:

1. Fill a supply bottle with deionized water. Connect the bottle to the washer or dispenser(Buffer valve "A" for Buffer Switching models).
2. Run the applicable DAY_RINSE protocol three times.
3. Perform your regular Overnight/Multi-Day Maintenance routine.

Weekly or As Needed use NaOH and HCl to remove proteins:

1. Flush the system with 0.1-0.5 N* NaOH (sodium hydroxide), followed by neutralization with an equivalent normality (0.1-0.5 N) of HCl (hydrochloride).
2. Rinse well with deionized water to remove the HCl.
3. Run the applicable DAY_RINSE protocol three times with deionized water if you plan to use the device immediately.

- * N = Normal solution, which contains 1 'gram equivalent weight' (gEW) of solute per liter of solution. The gram equivalent weight is equal to the molecular weight expressed as grams divided by the 'valency' of the solute.

Alternatively use an Enzyme-Active Detergent:

1. Mix an enzyme-active detergent according to the manufacturer's directions to fill a four-liter supply bottle. Connect the bottle to the washer's Buffer valve A or one of the Syringes. Connect a bottle of deionized or distilled water to Buffer valve B to rinse the tubing.
2. Run the **W-DECONTAMINATE** (W-DECON) or **S-DECONTAMINATE** protocol, as appropriate.
3. Respond to the Delay message, "Connect a bottle of water…", leave the detergent bottle connected and when ready, press **Continue**.
4. For the Syringes: connect a bottle with deionized or distilled water to the pump and REPEAT the protocol.
5. When the protocol is completed, connect a bottle containing four liters of deionized water and run W-DAY_RINSE or S-DAY_RINSE three times to flush the system.
6. Repeat the procedure for the other Syringe dispenser.

Periodic Maintenance

Periodic maintenance involves cleaning the components on a regular basis to keep the instrument running efficiently and in compliance with performance specifications. The recommended **frequency for cleaning components** is *at least monthly*. The risk and performance factors associated with your assays may require that some or all of the procedures be performed more frequently.

Warning! Internal Voltage. Turn off and unplug the instrument for all cleaning operations.

- **Important**: Do not apply lubricants to manifold O-rings, channel-end seals, bottle cover seals, any tubing connection, or any surface that is a part of the fluid path. The use of any lubricant on the fluid handling components will interfere with aspirate and dispense performance, and may cause irreparable damage to these components.

- **Water**: Daily maintenance is the key to keeping the liquid handler performing to specifications. In the maintenance procedures provided in this manual, the requirement to use distilled (dH2O) or deionized (DI) water can be met by numerous water purification methods, including MilliQ™. A minimum water purity of 2mOhm is expected.

Important!

- Do not immerse the instrument, spray it with liquid, or use a ”wet” cloth on it.
- Do not allow the cleaning solution to run into the interior of the instrument. (If this happens, contact the BioTek TAC.)
- Do not expose any part of the instrument to the recommended diluted sodium hypochlorite solution (bleach) for more than 20 minutes. Prolonged contact may damage the instrument surfaces.
- Be certain to rinse and thoroughly wipe all surfaces.
- Do not soak the keypad. Instead, moisten a clean cloth with deionized or distilled water and wipe the keypad. Dry it immediately with a clean, dry cloth.

Perform these preventive maintenance tasks regularly:

- Clean the Bottles on next page
- Clean the Plate Carrier on next page
- Washer Maintenance on page 181
- Peri-pump Dispenser Maintenance on page 188
- Syringe Dispenser Maintenance on page 193

Clean the Bottles

- Clean and rinse the supply bottles with deionized water before the first use, before each refill, and, periodically, as necessary, to prevent bacteria growth.
- Empty the waste bottle often (at least daily), and firmly seat the waste bottle stopper.
- Rinse the covers every time the wash or rinse bottles are filled.
- Accumulated algae, fungi, or mold may require decontamination.

> ▪ To ensure that fluid does not back up into the vacuum pump during operation, always operate the instrument with the **waste sensor cable** installed and the **waste detection sensor** enabled (the sensor is enabled by default).

If fluid collects in the **overflow bottle**, thoroughly rinse the level-switch assembly and bottle.

- Check the hex nuts securing the quick-disconnects to the bottle cap to ensure they are not loose or corroded.

Clean the Plate Carrier

If liquid has overflowed onto the plate carrier, transport rail, or glide strips, some buildup may occur and prevent the microplate from seating correctly on the carrier. This can interfere with plate transport. Weekly cleaning is recommended.

1. Turn the instrument off.
2. Lift the carrier up and off the transport rail.
3. Clean the carrier, rails, and glide strips, using mild detergent and hot water, 70% isopropyl alcohol, or ethanol. Clean the priming trough, too.
4. If detergent was used, wipe the components with a cloth moistened with water. Use a clean, dry cloth to dry the components.
5. Reinstall the carrier:
   - Place it on the transport rail so the slot on its base fits into the **Y-axis Carrier Arm**.
   - If necessary, release the spring-loaded microplate clamp in the back left corner of the carrier to level the carrier on the base.

Clean the Vacuum Filtration Carrier

Use a damp cloth to wipe up any spills, especially if the fluid is prone to dry and harden quickly. If necessary, flush it out with warm water by holding it under a running faucet for a few seconds.

Clean the exterior surfaces and mist shield

1. Turn off the instrument and disconnect the power cable.
2. Moisten a lint-free disposable towel with water, or with water and mild detergent. **Do not soak the cloth**.
3. Remove the mist shield if it is attached. Wipe the inside and outside surfaces of the mist shield with the towel. Wipe the top surface of the instrument base, and all exposed surfaces of the instrument.
4. If detergent was used, wipe all surfaces with a cloth moistened with water.
5. Use a clean, dry cloth to dry all wet surfaces.

Autoclavable Components

Autoclaving is an efficient method of sterilizing instrument components. For qualified items, it is a good alternative to some of the decontamination procedures.

Do autoclave:	Do NOT autoclave:
	Washer manifolds
Peri-pump cassettes	Plate carrier
16-Tube Syringe dispenser manifolds	32-Tube Syringe dispenser manifolds
Priming trough inserts	8-Tube Syringe dispenser manifolds (gray block holds two manifolds, 16 tubes total)
Autoclavable Syringe pump head (glass/stainless steel)	Non-autoclavable syringe pumps (black plastic)
Syringe module tubing with transparent amber check valves and glass bottles	Non-autoclavable syringe accessories, white check valves and plastic bottles

- Autoclaving the cassette typically increases the tubes' capacity. Expect the cassette to dispense more fluid than expected immediately after sterilizing or disinfecting the tubing. (When the cassette is completely dry, dispense volumes return to normal.)
- Autoclaving the dispense cassette does not diminish its expected life , **See Peri-Pump Dispenser on page 17**.

Washer Maintenance

The EL406's washer manifold, mist shield, and tubing require periodic cleaning.

Regular rinsing helps to keep the washer manifold clean, the aspiration and dispense tubes clear, and it increases the life of the tubing. Likewise, thoroughly cleaning the manifold to unclog aspirate and dispense tubes can correct poor or uneven aspiration or dispensing.

Washer Maintenance Tasks:

- Clean the manifold, mist shield, and tubing on page 179
- Create an AutoClean protocol on page 183 (when available). Run it regularly.
- Remove and clean the washer manifold on page 185
- Clean the Fluid Inlet Filter on page 186
- Maintaining the Vacuum Filtration System on page 187 (if applicable)

AutoClean the Washer

Some models of the EL406 feature BioTek's Ultrasonic Advantage™ for easy and thorough cleaning of the wash manifold. Instruments with AutoClean capability are easily identified by the stainless steel priming reservoir built into the instrument's base, under the wash manifold.

Warning! Ultrasonic energy is present in the cleaning reservoir when the AutoClean program is running. Do not put your fingers in the bath! Ultrasonic energy can harm human tissue.

- **Important!** Ensure there is adequate room in the waste bottle and sufficient volume in the supply bottle **before** running AutoClean!

AutoClean Procedure:

1. Empty the waste bottle.
2. Make sure supply bottles have sufficient volume of detergent and deionized water.
3. Select the predefined protocol, **W-CLEAN_w-BUFFER** or Create an AutoClean protocol on page 183.
4. Click **Run**.

5. Fill a supply bottle with deionized water and run **W-DAY_RINSE** one or two times to flush the system.

Tip: Detergent such as Terg-A-Zyme® added to deionized water in the supply bottle helps to break down the water's surface tension and enhances the cleaning process. Terg-A-Zyme also contains protease enzyme for assimilating protinaceous residue such as bovine serum albumin (BSA).

- Note: The **128-tube aspirate manifold** (1536-well hardware) does not completely evacuate the fluid from the trough. After the cleaning procedure is finished, you must manually absorb the remaining fluid (approximately 15 mL) with a pipette, paper towels or other method. Be sure to obey the manufacturer's handling guidelines when using detergents.

- AutoClean **32-Tube Dispense Manifolds**: With a little manual intervention, you can use the ultrasonic cleaner on the hard-to-clean dispense tubes, especially the small bore models. Find instructions in the operator's manual.

About AutoClean

BioTek's **Ultrasonic Advantage™** is a built-in **ultrasonic cleaner** that provides enhanced maintenance capabilities by using ultrasonic pulses in a water bath to clean the manifold tubes. Ultrasonic energy causes cavitation forces within the water bath, which in turn cause tiny vapor bubbles to be created. The formation and subsequent collapse of these bubbles is the mechanism that cleans the manifold tubes submerged in the bath.

The cleaner consists of a stainless steel reservoir with an ultrasonic transducer bonded to the bottom of the reservoir. The reservoir is mounted under the washer manifold and also functions as the priming trough.

- Do not try to remove the ultrasonic cleaner! Only BioTek authorized service personnel should remove the ultrasonic cleaner for maintenance or repair.

While the program is running, the ultrasonic cleaner will pulse on and off approximately every ten seconds, and you will hear a periodic hissing sound that indicates the ultrasonic energy is present.

- Not all EL406 models are equipped with this feature.

Create an AutoClean protocol

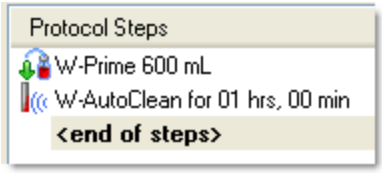
Create an **AutoClean** protocol for regular use. Make sure it begins by priming the tubing with the cleaning fluid. Approximately **93 mL** of fluid fills the reservoir during each run.

General Recommendation: Run **AutoClean** for 1 hour. Follow with a full prime using deionized water to remove the detergent from the system and/or with a wash buffer to leave the instrument primed and ready for use.

LHC	Keypad
W-AutoClean	DEFINE>CREATE>NAME>PLATE>ADD>WASHER→>ACLEAN

Define the cleaning protocol:

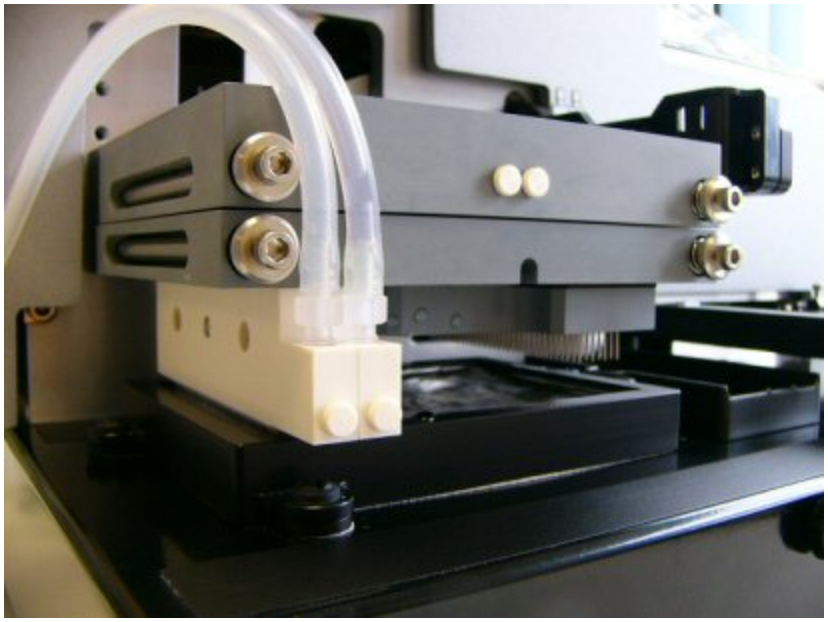
1. First, add a **Prime** step to fully prime the tubing with the cleaning fluid (or water).
2. Add an **AutoClean** step to the protocol.



3. Buffer Valve: select the supply bottle containing the desired cleaning fluid if Buffer Switching is installed.
4. Duration: enter the duration in hours and minutes; up to 24 hours.
5. Click **OK** to add a cleaning step to the protocol.

AutoClean the 32-Tube Dispense Manifold

With a little manual intervention, you can use the ultrasonic cleaner on the hard-to-clean dispense tubes, especially the SB – small bore models.



Before running AutoClean for the aspirate manifold, run it for the 32-tube Syringe dispenser manifolds:

1. First, remove the Peri-pump cassette, if applicable.
2. Grasp both Syringe dispenser manifolds, and keeping them together, slide them off the dispense arm. The magnets help keep them together.
3. Gently release their tubing from the black bracket above the wash manifold and position them on the left side of the wash manifold, ready to put into the ultrasonic bath.
4. Run the AutoClean protocol: Create an AutoClean protocol on previous page.
5. When AutoClean begins, lift the wash manifold with your right hand and place the dispense manifolds into the bath with your left hand.
6. Make sure the tubes are in the fluid and the edges of the manifolds are resting on the edges of the priming trough. Then, gently lower the wash manifold down on top of the dispense manifolds. The wash manifold will hold them in place.

   - Important: the aspirate manifold will be unable to evacuate the fluid from the priming trough when the run ends because the dispense manifolds will be in the way. To complete the process:

7. Reinstall the dispense manifolds on the dispense arm.
8. Run the AutoClean protocol for the aspirate manifold.

9. When AutoClean is finished, remove the residual liquid in the priming trough using a paper towel or other method. Be sure to follow the manufacturer's handling instructions for the cleaning fluid you are using.

Remove and clean the washer manifold

- **DO NOT AUTOCLAVE** the manifold!

1. Run the system "dry" to remove any fluid: Connect an empty supply bottle and run a prime protocol until the tubing is empty.
2. Turn off the instrument, disconnect the power cable and remove the mist shield.

- Dual manifolds: Hold the two manifolds together as a single unit when removing and reinstalling.

3. Carefully remove the manifold(s) and end plates, if applicable.

Avoid pressing the stylus against the sides of the tubes during cleaning. This can cause the tubes to bend, adversely affecting dispense precision.

4. Using a soft-bristled brush, thoroughly clean the outside of the manifolds. Clean the insides of each tube with the appropriate stylus (aspirate/dispense). Flush hot water through the cross channels.
5. Rinse the manifold with deionized or distilled water. Check to see if water comes out of all dispense and aspirate tubes. If not, soak the manifold in hot, soapy water and repeat.

Caution. When reinstalling the manifold, only tighten the screw-washer-spring assembly that holds it in place until you feel the mechanical stop. Tightening past this point will damage the instrument and will **void your warranty.**

6. When satisfied, reassemble the manifold and end plates, making sure that the two o-rings are in place prior to reassembly. **Do not overtighten the manifold screws.**
7. Install the mist shield.
8. Reconnect the power cable and turn on the instrument.
9. Prime the system with deionized water by running **W-DAY_RINSE** or a similar Maintenance or Prime protocol. Watch for leaks. If fluid leaks out of the back of the instrument, firmly seat the tubing. If fluid leaks from the manifold, try disassembling and carefully reassembling.

10. Verify aspirate/dispense performance visually or by performing the qualification tests.

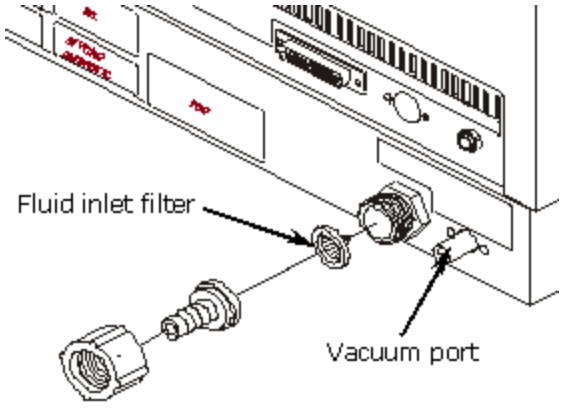
- Replace the O-rings on an annual basis. Replace the channel-end seals (rubber plugs) if they show signs of cracking or drying out. See Replace Washer Manifold O-rings and Seals on page 208.

Clean the Fluid Inlet Filter

Warning! Internal Voltage. Turn off and unplug the instrument for all cleaning operations.

Periodically clean the fluid inlet filter (PN 49943):

1. Unscrew the **Dispense Fluid In** fitting from the back of the instrument.
2. Note the orientation of the filter in the fitting (the cone-shaped end of the filter points "in" toward the instrument) and then remove the filter from the fitting.
3. Wash the filter with hot water and a soft-bristled brush.
4. Rinse the filter, then replace it in the fitting and reinstall.



Maintaining the Vacuum Filtration System

Perform the following tasks as part of your regular maintenance when you are using the vacuum filtration system:

Clean the Vacuum Filtration Carrier

Use a damp cloth to wipe up any spills, especially if the fluid is prone to dry and harden quickly. If necessary, flush it out with warm water by holding it under a running faucet for a few seconds.

- Decontaminate the Vacuum Filtration System on page 204
- Vacuum Filtration Carrier: Replace the Gasket on page 210 (as needed)

Peri-pump Dispenser Maintenance

The level of the maintenance required to keep the dispenser performing as expected is highly dependent on several factors, including the type of fluid dispensed, the frequency of dispensing, and the work habits employed. For example, when dispensing fluids that can crystallize or harden after use, maintenance activities are required more frequently.

 When using the 1 µL cassette filter fluids to 50 microns to reduce the chance of tips clogging.

Daily maintenance includes purging the fluid at the end of a dispense run and flushing the tubing with water (or buffered saline and then water). This is a good practice whenever the dispenser will be idle for more than an hour, as well as at the end of the day.

Another important daily requirement is keeping track of the number of plates processed with a cassette. This is necessary to determine when the cassette has reached its expected lifetime and is due for replacement or recalibration. Replacement Tubing Kits, a refurbishment service, and new cassettes are available from BioTek Instruments.

Monthly maintenance requires overall cleaning of the dispenser and its accessories, and verifying performance to determine if the cassette needs recalibration. Autoclaving or decontaminating the cassette is also recommended.

Peri-pump Maintenance Tasks:

- Flush the Dispense Cassette on the facing page
- Unclog the Dispense Tips on page 190
- Record the Number of Plates Processed on page 191
- Recalibrate the Peri-pump Dispense Cassette on page 211
- Replace Peri-pump Dispense Cassette Tubing on page 210

Flush the Dispense Cassette

Prime the tubing with an appropriate reagent at the beginning of the day, and, flush the tubing to effectively remove all contaminants at the end of the day.

The type of rinse fluid to use is determined by the type of fluid you are dispensing. Some dispense fluids require the use of enzyme-active detergent, buffered saline, ethanol or isopropyl alcohol, rather than deionized water alone.

Tools and Supplies

- Deionized or distilled water
- Buffered saline solution or enzyme-active detergent for protein or cell based assays

At the start of the day:

Prime the tubing to prepare for a dispense run.

1. Reload the cassette and fill the supply vessel:
   - When dispensing solutions not effected by water, simply prime with the dispense fluid.
   - When dispensing protein solutions, first prime the tubing with a buffered saline solution to remove any traces of water in the tubing, then, prime with the dispense fluid.
2. **Prime** the tubing until fluid flows into the priming trough and all visible air bubbles have been removed.

At the end of the day:

Purge the tubing to reclaim the dispense fluid, then Prime the tubing to flush it clean.

1. **Purge** the cassette until the tubing appears empty.
2. Replace the supply vessel with the appropriate rinse fluid:
   - When dispensing water soluble solutions use deionized or distilled water.
   - When dispensing protein solutions, first prime the tubing with a buffered saline solution to remove protein particles, then, prime with deionized or distilled water.
3. **Prime** the tubing for the specified duration:
   - 1 µL cassette = 5 seconds

- 5 µL cassette = 7 seconds
- 10 µL cassette = 10 seconds.

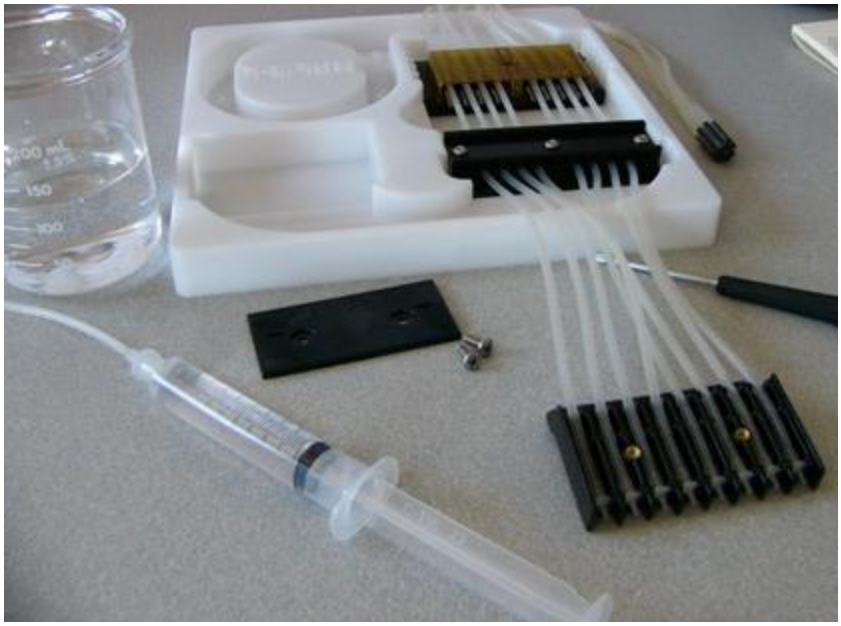
Unclog the Dispense Tips

The small diameter of the dispense tips makes them susceptible to clogging. You may be able to visually identify a clogged tip, or inaccurate dispense performance may signal a problem. Good work habits can prevent clogging or reduce its occurrence:

- When using 1 µL cassettes, filter fluids to 50 microns before dispensing.
- Thoroughly flush the tubing after/in-between usage, especially when using liquids that crystallize or harden.

In case the need arises, BioTek ships a 10 cc plastic syringe with special tubing and fitting for use unclogging tips. Installation instructions recommend storing it in the pouch on the back of the instrument. The remedy involves removing the dispense tip and flushing it with water. Depending on the type of clog, soaking the tip holder in hot water with mild detergent is recommended.

 This task may be easier if you use the cassette's shipping container to hold the unaffected cassette parts, keeping them out of your way.



Required Materials

- 10 cc syringe with tubing and fitting attachment shipped with dispenser
- Screwdriver shipped with dispenser

- A sufficient quantity of deionized (DI) water in a beaker

Procedure

1. Fill the 10 cc syringe with water and set aside.
2. Remove the cassette from the dispenser.
3. Use the screwdriver to open the **Tip Holder**. Put the top of the holder aside.
4. Lift the affected dispense tube from the holder and pull its tip off the tube.
5. Slide the tip, tapered end first, into the tubing on the end of the syringe.
6. With the tip poised to expel the clog and the water into the beaker or a sink, discharge the syringe.
7. Fill and discharge the syringe as many times as needed to flush the tip.
8. Reassemble the cassette:

- Put the straight end of the tip into the bottom of the tube (the tapered end of the dispense tip is exposed).
- Reinsert the tube into the Tip Holder. Seat the flared edges of the tip into the molded slots.
- Replace the Tip Holder cover with its two screws. The etched BioTek label identifies the top of the cover (except for 1536 cassettes' steel cover plate).

Record the Number of Plates Processed

To determine when a tubing cassette has reached the end of its expected lifetime, make a habit of counting and recording the approximate number of plates and volume dispensed per cassette.

Create a form similar to the example table below or estimate your usage of the cassette and project a date for replacement or recalibration.

Cassette Expected Lifetime

Cassette Types	Cassette Life	Total Volume
1 µL	1000 384-well plates @ 5 µL/well	2,000 mL
5 µL	1000 96-well plates @ 50 µL/well	5,000 mL
10 µL	1000 96-well plates @ 100 µL/well	10,000 mL

With strict adherence to best practices and maintenance recommendations, this is the typical longevity of the dispense cassettes.

Example table for recording cassette usage:

You may want to create a form similar to this table to keep track of the volume dispensed with each cassette:

Cassette serial #: **2178**					
Date	# Plates	Plate Type	Volume/Well	Total Daily Vol.	Total Cassette Vol
10/10/08	26	384	5 µL	49920 µL	50 mL
10/11/08	33	96	10 µL	31680 µL	82 mL

Syringe Dispenser Maintenance

The level of the maintenance required to keep the dispenser performing as expected is highly dependent on several factors, including the type of fluid dispensed, the frequency of dispensing, and the work habits employed. For example, when dispensing fluids that can crystallize or harden after use, maintenance activities are required more frequently.

32-tube small bore (SB) dispense manifold: If the fluid streaming from a dispense tube appears to be awry or skewed, it is most likely caused by minute particles of debris on the end of the tube. Brush away any particles from the end of the tube using a piece of silicon tubing. Silicon will not flake off and leave particles behind like other materials.

See AutoClean the 32-Tube Dispense Manifold on page 183

Clean the Bottles and Tubes

- Clean the dispense and rinse bottles and supply tubes with deionized water before the first use, before each refill, and if they have been idle for any length of time.
- Accumulated algae, fungi, or mold may require decontamination.

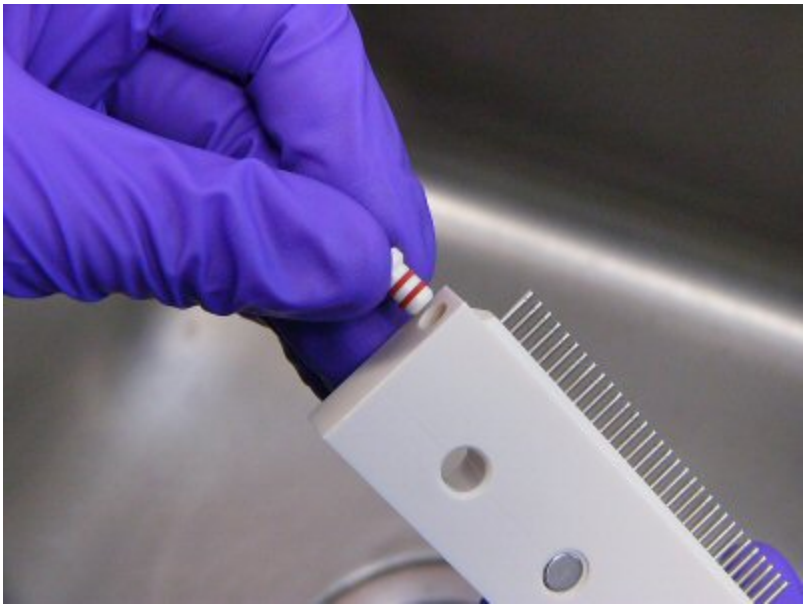
Syringe Dispenser Maintenance Tasks

- Autoclave the Syringe Head on page 196
- Clean the Dispense Tubes on page 195
- Clean the Syringe Dispenser Manifold below
- Clean or Replace the Check Valves on page 196
- Run **AutoPrime** to soak the dispense tubes

Clean the Syringe Dispenser Manifold

Regular rinsing helps to keep the manifold clean and the dispense tubing clear, and will increase the life of the tubing. Follow the **Decontamination** procedure to disinfect the manifold and tubing.

If you suspect a particular problem is related to the manifold (for example, clogged tubes can result in uneven dispensing), you should perform a thorough cleaning of the manifold.



To clean the manifold:

1. Turn off and unplug the instrument.
2. Pull the manifold off of the dispense arm and disconnect the tubing from the manifold.
3. Remove the plugs from the ends of the manifold. Using a lint-free disposable towel, thoroughly clean the outside of the dispense tubes.
4. Run hot water through the inlet fitting. Check to see if water comes out of all of the dispense tubes. If not, soak the manifold in hot soapy water and repeat. Clean the Dispense Tubes on the facing page, if necessary.

Clean the Dispense Tubes

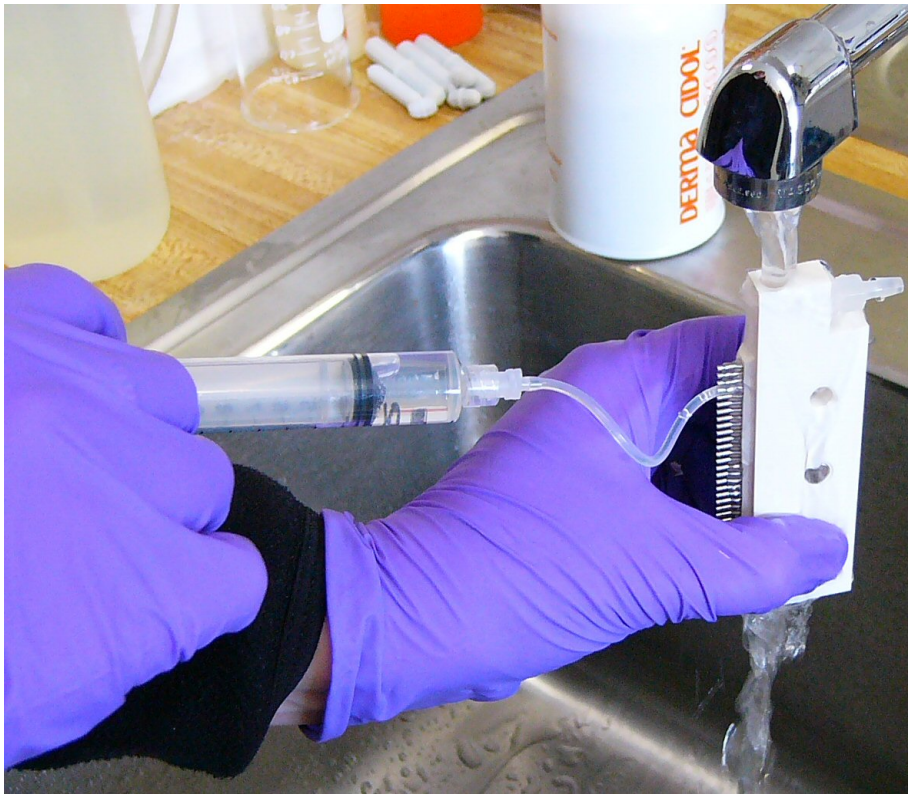
- **Do NOT autoclave the dual 8-tube and 32-tube dispense manifolds!**

- Note: The autoclavable 16-tube and non-autoclavable 8-tube manifolds have removable dispense tubes. We do not recommend routinely removing these tubes. In the case of a particularly difficult problem with any one channel, however, a tube may be removed and cleaned individually, or replaced.

Clean the Tubes

Unless there is a problem, the manifold dispense tubes do not need special cleaning. Periodic rinsing is usually sufficient to keep the tubes clean. However, if the regular maintenance is not completely successful, try the following:

- 8- and 16-tube manifolds: clean the tubes with the stylus;
- 32-tube manifolds: flush the tubing with the 10 mL syringe.



1. Remove the plugs from the ends of the manifold.
2. Tip the manifold on end and flush hot water through this open channel.
3. Using the supplied tool, clean the insides of all of the dispense tubes.

- 8- and 16-tube manifold stylus: PN 2872304
- 32-tube manifold: 10 cc syringe and tubing

Let water flow through the open channel while you probe or flush each tube, forcing any particles to be washed away.

4. Rinse the manifold with deionized or distilled water. Check to see if water comes out of all of the dispense tubes (except when working with the 32-tube SB manifold). Reinsert the plugs into the ends of the manifold.
5. Remount the manifold and replace the tubing.
6. Run a Prime protocol using 40 mL of deionized water.
7. Verify dispense performance visually, or Perform the Syringe Dispense Precision & Accuracy Test on page 262.

Clean or Replace the Check Valves

- The check valves do not twist open.

If the check valves leak or become clogged, you can either clean or replace them. Contact BioTek Customer Service to order replacement check valves.

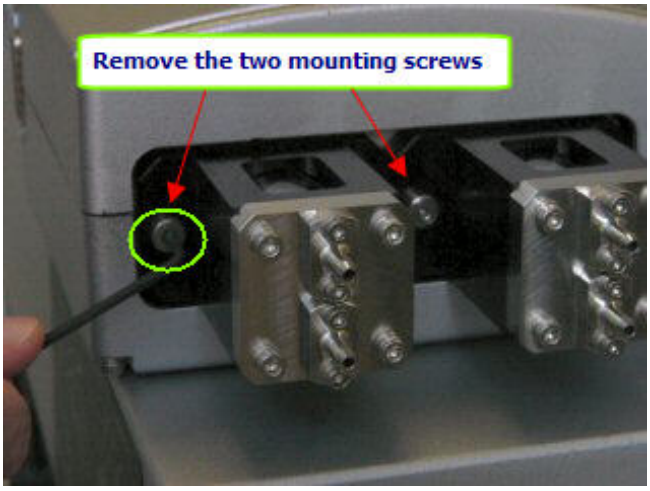
To clean a check valve:

1. Pull the tubing off the check valve.
2. Insert the stylus into the feed end of the valve to hold it open (observe arrow on valve indicating flow direction).
3. Flush with hot water.
4. Replace the valve and the tubing.

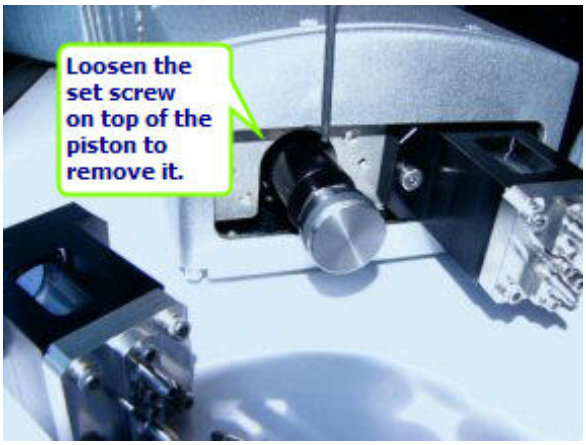
Replace Syringe Dispenser Check Valve on page 211, if necessary.

Autoclave the Syringe Head

Certain models of the Syringe dispenser are ***not*** autoclavable. Be sure your dispenser is described as autoclavable before proceeding. **See Syringe Dispenser-Autoclavable vs. Non-autoclavable on page 147**.



1. Use the supplied 3/32″ (2.39 mm) hex wrench to remove the two mounting screws that hold the syringe head in the unit.
2. Pull the syringe head straight back and off of the piston



3. Use the hex wrench to loosen the setscrew on top of the sleeve that holds the piston and then remove the piston.

Important! Autoclave the piston and syringe head separated from one another. Keep the piston and syringe head unattached to each other when autoclaving.

4. Autoclave at 134°C and 216 kPa for 3 minutes, or 121°C and 115 kPa for 30 minutes. The manifold, tubing, autoclavable check valves, and supply bottles may also be sterilized in the autoclave.

- Check valves (PN 68073) recommended for use with organic substances **cannot** be autoclaved.

5. Replace the components by reversing the steps:
6. With the flat side of the shaft facing up, slide the syringe piston shaft into the piston holder until it stops.
7. Use the 3/32″ (2.39 mm) hex wrench to tighten the setscrew.
8. Push the syringe head over the piston until it is flush with the unit and use the hex wrench to attach the two mounting screws.

Decontamination

Any laboratory instrument that has been used for research or clinical analysis is considered a biohazard and requires decontamination prior to handling.

Decontamination minimizes the risk to all who come into contact with the instrument during shipping, handling, and servicing. Decontamination is required by the U.S. Department of Transportation regulations. Persons performing the decontamination process must be familiar with the basic setup and operation of the instrument.

The recommended **frequency for decontamination** is at least monthly, and before shipment of the instrument to BioTek for calibration or repair.

- **Important!** BioTek Instruments, Inc. recommends the use of the following decontamination solutions and methods based on our knowledge of the instrument and recommendations of the Centers for Disease Control and Prevention (CDC). Neither BioTek nor the CDC assumes any liability for the adequacy of these solutions and methods. Each laboratory must ensure that decontamination procedures are adequate for the biohazards they handle.

Warning! Internal Voltage. Turn off and unplug the instrument for all decontamination operations.

- **Do not** immerse the instrument, spray it with liquid, or use a "wet" cloth. Do not allow the cleaning solution to run into the interior of the instrument. If this happens, contact the BioTek TAC. **Do not soak the keypad.**

- **Wear prophylactic gloves** when handling contaminated instruments. Gloved hands should be considered contaminated at all times; keep gloved hands away from eyes, mouth, nose, and ears. Eating and drinking while decontaminating instruments is not advised.

- Mucous membranes are considered prime entry routes for infectious agents. Wear eye protection and a surgical mask when there is a possibility of aerosol contamination. Intact skin is generally considered an effective barrier against infectious organisms; however, small abrasions and cuts may not always be visible. Wear protective gloves when performing the decontamination procedure.

Tools and Supplies

0.5% sodium hypochlorite (NaClO, or bleach)

70% isopropyl alcohol (as a bleach alternative)
Deionized or distilled water
Priming plate
Safety glasses
Surgical mask
Protective gloves
Lab coat
Biohazard trash bags
Clean cotton cloths

Step-by-Step Decontamination Instructions:

- Decontaminate Exterior Surfaces below
- Decontaminate Tubing and Manifold on page 202
- Alternate Decontamination Procedure for Tubing and Manifold on page 203

Decontaminate Exterior Surfaces

Caution! Be sure to check the percentage NaClO of the bleach you are using; this information is printed on the side of the bottle. Commercial bleach is typically 10% NaClO; in this case, prepare a 1:20 dilution. Household bleach is typically 5% NaClO; in this case, prepare a 1:10 dilution.

The bleach solution is caustic; wear gloves and eye protection when handling.

1. Turn off the instrument and disconnect the power cord. Empty the waste bottle.
2. Unload the Peri-pump cassette and the prime trough inserts, and remove the Syringe dispenser manifold and tubing, if applicable.
3. Autoclave the cassette and other autoclavable components.
4. Prepare an aqueous solution of 0.5% sodium hypochlorite (NaClO, or bleach). As an alternative, 70% isopropyl alcohol (or 70% ethanol) may be used if the effects of bleach are a concern.

   - **Isopropyl alcohol** is not recommended for removing **proteins** (such as bovine serum albumin).

5. Moisten a cloth with the bleach solution or alcohol. **Do not soak the cloth**.

- Wipe the keypad (do not soak). Wipe again with a clean cloth moistened with deionized or distilled water. Dry immediately with a clean, dry cloth.
- Remove the mist shield if it is attached. Wipe the inside and outside surfaces of the mist shield.
- Wipe the plate carrier, top surface of the instrument's base, supply bottles and tubing, and all exposed surfaces of the instrument.

6. Wait 20 minutes. Moisten a cloth with DI or distilled water.
   - Wipe the inside and outside surfaces of the mist shield.
   - Wipe the plate carrier, top surface of the instrument's base, supply bottles, tubing, bottle covers and all exposed surfaces of the instrument that have been cleaned with the bleach solution or alcohol.
7. Use a clean, dry cloth to dry all wet surfaces.
8. Reassemble the instrument as necessary.
9. Discard the used gloves and cloths using a biohazard trash bag and an approved biohazard container.

Decontaminate Tubing and Manifold

Predefined protocols to flush and soak the supply tubing and manifolds with disinfectant, then flush the system with rinse fluid are installed onboard the instrument and on the host computer during installation of the LHC:

- **W-DECONTAMINATE** for the washer
- **S-DECONTAMINATE** (S-DECON onboard) for the Syringe dispenser

For the Syringe dispensers: copy this protocol and modify it to run on Syringe B.

When storing or shipping the instrument, the **LONG_SHUTDOWN** procedure (on 1) primes and soaks the instrument, and ends by pushing air through the system. The parameters can be edited for optimum cleaning. For example, consider using ethanol instead of air to complete the decontamination process.

- Two supply bottles are required for this procedure: one for disinfectant, and one for rinse.

1. Empty the waste bottle.
2. Prepare an aqueous solution of 0.5% sodium hypochlorite (NaClO, or bleach).
3. Fill one supply bottle with at least 400 mL of bleach solution (disinfectant).
4. Fill another supply bottle with at least 800 mL of deionized water (rinse).

   When using **Buffer Switching**:

   - Buffer valve A: Disinfectant
   - Buffer valve B: Rinse solution (they will be reversed in the next round)

   Without Buffer Switching, connect the bottle containing disinfectant to the **Fluid In** port.
5. Reconnect the power cord and turn on the instrument.
6. Run the decontamination protocols.

Preparing to run **W-DECON** or **W-DECONTAMINATE**:

Keypad with Buffer Switching	Connect the supply bottles this way: • Buffer valve A: Disinfectant bottle • Buffer valve B: Rinse solution bottle
Keypad without Buffer Switching	Connect the disinfectant bottle to the Fluid In port. When the protocol is finished, connect the rinse bottle to the inlet

	port, and press REPEAT to rerun the protocol. **Keypad**: Run **DECON_STEP1** followed by **DECON_STEP2**. **LHC**: At the prompt/delay, change bottles.
LHC with Buffer Switching	Connect the supply bottles this way: • Buffer valve A: Disinfectant bottle • Buffer valve B: Rinse solution bottle For unattended operation, change the Delay steps, make the delay a fixed time rather than indefinite.
LHC without Buffer Switching	Connect the disinfectant bottle to the Fluid In port. At the prompt, when the protocol is delayed, connect the rinse bottle to the inlet port, and press Continue to complete the protocol.

Preparing to run S-DECON or S-DECONTAMINATE:

- Buffer Switching for the Syringe dispenser: modify the predefined protocols to take advantage of the buffer switching module.

Using the	Description
Keypad	Connect the disinfectant bottle to the Syringe A port. When the protocol is finished, connect the rinse bottle to the port, and press REPEAT to rerun the protocol. Make a copy of the protocol and modify the copy for Syringe B, assign an unique name, e.g. SB-DECON, and repeat the above procedure.
LHC	Connect the disinfectant bottle to the Syringe A port. At the prompt, when the protocol is delayed, connect the rinse bottle to the inlet port, and press Continue to complete the protocol. Copy the protocol, select File>Save As, assign it a unique name, e.g. SB-DECONTAMINATE, and modify it for Syringe B. Then, replicate the procedure.

See also Alternate Decontamination Procedure for Tubing and Manifold below.

Alternate Decontamination Procedure for Tubing and Manifold

If you are unable to run the decontamination protocols due to a system failure, perform the following alternate decontamination procedure to disinfect the internal tubing and manifolds.

Caution! Be sure to check the percentage NaClO of the bleach you are using; this information is printed on the side of the bottle. Commercial bleach is typically 10% NaClO; in this case, prepare a 1:20 dilution. Household bleach is typically 5% NaClO; in this case, prepare a 1:10 dilution.

1. Turn off the instrument and disconnect the power cord.
2. Remove the mist shield.

3. Using the 9/64" (3.57 mm) hex wrench, remove the screws, washers, and springs that hold the washer manifolds in place. Remove the wash manifold(s) and end plate(s).

   - For dual manifold models, hold the two manifolds (and end plates) together as a single unit when removing and replacing them.

4. Prepare an aqueous solution of 0.5% sodium hypochlorite (bleach). As an alternative, 70% isopropyl alcohol (or ethanol) may be used if the effects of bleach are a concern.

   - **Isopropyl alcohol** is not recommended for removing **proteins** (such as bovine serum albumin).

5. Soak the tubing and manifold in the bleach or alcohol solution.
6. Wait 20 minutes. Rinse the tubing and manifold with DI or distilled water.
7. Use a clean, dry cloth to dry all wet surfaces of the tubes and manifold.
8. Reassemble the washer manifold, making sure that the o-rings/gaskets are in place prior to reassembly. **Do not over tighten the manifold screws.**
9. Re-attach the mist shield.
10. Prime the system by running **W-DAY_RINSE** or a similar Maintenance or Prime protocol. Watch for leaks. If fluid leaks out of the back of the instrument, firmly seat the tubing. If fluid leaks from the manifold, try disassembling and carefully reassembling.
11. Discard the used gloves and cloths using a biohazard trash bag and an approved biohazard container.
12. Verify performance visually or by performing qualification tests.

Decontaminate the Vacuum Filtration System

First, disinfect the tubing, then, remove and soak the carrier:

Decontaminate the tubing:

Run the predefined protocol **VAC30/10_TEST** (depending on your instrument model) with disinfectant as the fluid supply to clean the tubing and hardware used to perform filter plate assays.

1. Connect a supply bottle filled with 200 mL of bleach solution/disinfectant.
2. Insert the black vent plug into the vent port on the front of the carrier.

3. First prime the tubing.
4. Run **TEST**.
5. When the run is completed, replace the decontamination fluid supply with deionized or distilled water and rerun it once or twice.

Decontaminate the vacuum filtration carrier:

1. Turn off and unplug the instrument.
2. Disconnect the tubing from the vacuum carrier.
3. Remove the carrier and soak it in the Decontamination (bleach) solution for 20 minutes.
4. Rinse the carrier thoroughly to remove all bleach.
5. Use a clean, dry cloth to dry all wet surfaces.

Inspect the plate seal gasket and replace it if defects are observed replace the gasket: Vacuum Filtration Carrier: Replace the Gasket on page 210.

Long Shutdown (Prepare for Storage or Shipment)

Before the EL406 is shipped or stored, the entire system should be rinsed and soaked with disinfectant and then purged of all fluid. Perform these steps when leaving the instrument unused for a long period of time.

Predefined protocols are installed onboard the instrument and on the host computer during installation of the LHC:

- **W-LONG_SHUTDOWN** — for the washer
- **S-LONG_SHUTDOWN** — for the Syringe dispenser

The LONG_SHUTDOWN protocols flush and soak the supply and manifold tubing with disinfectant, then flush with rinse, and finally purge the system of fluid.

For the Syringe dispensers: copy this protocol and modify it to run on Syringe B.

- Three supply bottles are required for this procedure: one for disinfectant, one for rinse, and one for air.

Caution! Be sure to check the percentage NaClO of the bleach you are using; this information is printed on the side of the bottle. Commercial bleach is typically 10% NaClO; in this case, prepare a 1:20 dilution. Household bleach is typically 5% NaClO; in this case, prepare a 1:10 dilution.

1. Turn the instrument off and disconnect the power cord.
2. Unload the Peri-pump cassette and the prime trough inserts, if applicable. Clean and store them separately.
3. Empty the waste bottle.
4. Prepare an aqueous solution of 0.5% sodium hypochlorite (NaClO, or bleach).
5. Fill one supply bottle with at least 400 mL of bleach solution (disinfectant).
6. Fill another supply bottle with at least 800 mL of deionized water (rinse).
7. Keep the third supply bottle empty (air).

   When using **Buffer Switching** , modify the protocol to correspond and connect the supply bottles this way:

   - **Valve A:** Disinfectant bottle
   - **Valve B:** Rinse solution bottle
   - **Valve C:** Empty bottle

Without Buffer Switching, connect the bottle containing disinfectant to the **Fluid In** port, and obey the prompts to change bottles.

8. Turn on the instrument and run **W-LONG_SHUTDOWN**.

> ▪ While this program is running, you will need to periodically check the display panel and follow the instructions.

9. When the washer has been cleaned and purged, repeat the process to clean and purge the Syringe dispensers, if applicable:
   - Prepare the supply bottles with disinfectant, rinse and air;
   - Run **S-LONG_SHUTDOWN**

Storing the Instrument

After performing the Long Shutdown (Prep for Storage or Shipment) on previous page protocols:

- Turn off the instrument and disconnect the power cord.
- Store it on a flat surface that is relatively free of vibration, in a dust-free and particle-free environment.
- Protect the instrument from temperature extremes that can cause condensation within the unit and from corrosive fumes and vapors.
- Store the instrument under the following environmental conditions:

Temperature:	20° to 50°C (-4° to 122°F)
Relative humidity:	10% to 85% (non-condensing)

> ▪ Important: Allow the instrument to reach room temperature before use after storage.

Replace Components

Some components of the EL406 must be replaced periodically to maintain specified performance levels.

Washer Components:

- Replace the Vacuum Pump Fuse on next page
- Replace Washer Manifold O-rings and Seals on next page

- Clean the Fluid Inlet Filter on page 186
- Vacuum Filtration Carrier: Replace the Gasket on page 210

Peri-pump Components

- Replace Peri-pump Dispense Cassette Tubing on page 210
- Recalibrate the Peri-pump Dispense Cassette on page 211

Syringe Dispenser Components

- Clean or Replace the Check Valves on page 196
- Calibrate the Backlash for Syringe Dispenser on page 212

Replace the Vacuum Pump Fuse

Spare fuses (PN 46055) are shipped with the instrument in case the pump blows a fuse.

Tools: Screwdriver

To change the fuse:

1. Locate the **Accessory Fuse** port on the rear panel below the **Accessory Outlet** for the vacuum pump.
2. Use a screwdriver to open the port and release the fuse. It has a spring action.
3. Replace the fuse and reinstall.

Replace Washer Manifold O-rings and Seals

> ▪ This is **not** applicable to the 128-tube aspirate manifold. The plugs in its manifold are the same as those used in the Syringe dispenser manifolds: PN 45090.

For optimal performance and to extend the life of the washer, replace the manifold O-rings once a year and the channel-end seals (rubber plugs) when they show signs of cracking or drying out.

Order the replacement O-rings and seals from BioTek:

- O-rings: PN 49941
- Channel-end seals: PN 49486

You must remove the washer manifold to change the O-rings and seals, so these tasks work best in conjunction with the regular monthly maintenance routines.

Replacing the O-Rings

After cleaning and rinsing the manifold:

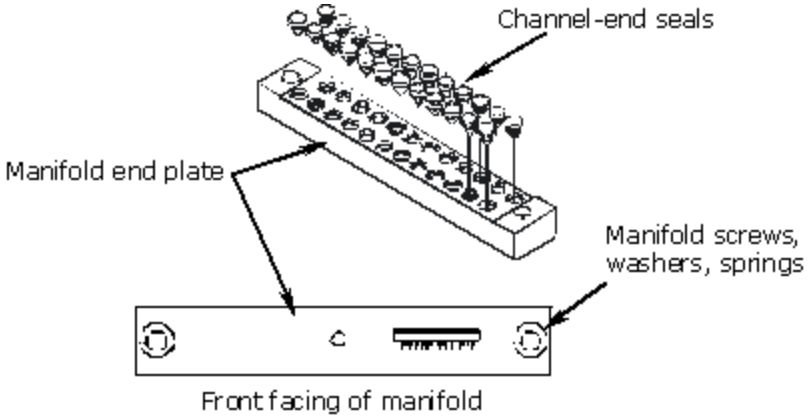
1. Using your fingers or an appropriate tool, such as a very small, flat screwdriver, remove the two O-rings that are exposed when the manifold is removed.
2. Replace the used O-rings with new ones by fitting them into the grooved slots.
3. If you are NOT replacing the channel end-seals at this time, reinstall the manifold. **Do not over-tighten the manifold screws.**

Caution: When reinstalling the manifold, only tighten the screw-washer-spring assembly that holds it in place until you feel the mechanical stop. Tightening past this point will damage the instrument and **void your warranty.**

Replacing/Cleaning the Channel-End Seals

If the channel-end seals do not need to be replaced, they should be washed with mild detergent or alcohol.

1. Remove the manifold end plates to access the channel-end seals.



The manifold end plate sits in front of the manifold and holds the screws, washers, and springs that hold the manifold in place. The channel-end seals sit in bored holes in the backside of the end plate, facing the manifold's channels. The manifold end plate has markings to indicate its position relative to the manifold, e.g., Top or Bottom, on dual manifolds.

2. Using an appropriate tool, such as a very small, flat screwdriver or a paper clip, remove the seals from the manifold end plate.

Caution: Do not grease any parts of this mechanism. Lubricate the seals and the bored holes with alcohol to assist with reinsertion.

3. Clean the seals if you are not replacing them with new ones.
4. Lubricate both the seals and the bored holes with 70% isopropyl alcohol to facilitate insertion of the seals. Make sure the seals sit firmly in the bored holes in the manifold end plate.

   - The 9/64" (3.57 mm) hex wrench shipped with the washer is useful for reinserting the seals into the bored holes.

5. When all of the seals are in place, reinstall the manifold end plates and the manifold. **Do not over-tighten the manifold screws**.

Vacuum Filtration Carrier: Replace the Gasket

If you observe damage to the vacuum filtration carrier's gasket, you must order a replacement part from BioTek, and follow these instructions to replace it.

Tools: Philips head screwdriver

1. Remove the carrier from the instrument and put on a level work surface.
2. With a Philips screwdriver, remove the six screws around the perimeter of the top of the carrier.
3. Lift off and set aside the top plate, and remove the gasket.
   - If the grate (located under the gasket) needs to be cleaned, rinse it under tap water. Allow the grate to dry before placing it and the gasket in the carrier.
4. Place the new gasket in the carrier, correctly positioning the notch with the corresponding indentation on the carrier's right side.

   - **Note:** The gasket fits only one way on the carrier. When replacing it, align the notch in the gasket with the small "bump" on the carrier.

5. Restore the top plate and its six screws. Do not overtighten.
6. Verify vacuum filtration performance by performing the Qualification procedure: Vacuum Filtration Evacuation Efficiency Test on page 237.

Replace Peri-pump Dispense Cassette Tubing

BioTek provides replacement tubing kits as an alternative to buying a new cassette. Purchase the replacement tubing kits from BioTek and follow the instructions shipped with the kit or on the EL406 Operator's Manual CD in the "Cassette Calibration" folder, titled: **7171017_(*current Rev*)_Replacing the tubing_**

8x14.PDF. For the best experience with these instructions print them on legal size paper (8½" x 14").

Recalibrate the Peri-pump Dispense Cassette

Calibration Kit

BioTek offers an accessory for recalibrating dispense cassettes. The Calibration Kit (PN 7170017) speeds up the recalibrating process and is useful for verifying performance.

Follow the instructions shipped with the kit or find them on the EL406 Operator's Manual CD in the "Cassette Calibration" folder, titled: **7171009_(*current Rev*)_ Calibration Kit Instructions.PDF**.

Gravimetric Method

The alternative and most precise method for calibrating a cassette is the gravimetric method. Find the instructions on the EL406 Operator's Manual CD in the "Cassette Calibration" folder: it is titled: **7171024_(*current Rev*)_Calibrating Gravimetrically.PDF**.

Replace Syringe Dispenser Check Valve

You can order replacement check valves from BioTek Customer Care if your check valves become clogged and cleaning them does not solve the problem:

- PN 68083 — Autoclavable valves for use with non-organic substances.
- PN 68073 — Check valves recommended for use with organic substances.

> ▪ If you observe a decline in performance after changing the check valves, Calibrate the Backlash for Syringe Dispenser on next page.

Calibrate the Backlash for Syringe Dispenser

If you have replaced a check valve or installed the Buffer Switching module and subsequently noticed a decline in performance, recalibrating the backlash is recommended to restore accuracy in dispense volumes.

Equipment Required

- Microplates: 384-well plates for testing the 16-tube dispensers (which can be replaced with 96-well plates if more applicable for your lab); 1536-well plates for testing the 32-tube dispensers; and 96-well plates for testing the 8-tube dispensers.
- Precision balance with minimum capacity of 100 g and readability of 0.001 g resolution
- Supply bottle with deionized water

Setup

While calibrating and testing, try to maintain a steady liquid level in the supply bottle, keeping it half full. Start with more fluid to allow for priming. Connect the supply bottle to the Syringe under test. Make sure the supply bottle is at the same level as the dispenser.

Create a Dispense Protocol

Create a protocol that dispenses the correct volume for the manifold under test:

- 16-tube manifold: **20 µL** per well to a **384-well** plate at Flow Rate **2**.
- 32-tube manifold: **6 µL** per well to a **1536-well** plate at Flow Rate **2**.
- 8-tube manifold: **20 µL** per well to a **96-well** plate at Flow Rate **2**.

Prime and Dispense

1. Run a Prime protocol, for example, **S-DAY_RINSE** or **S-DAY_RINSE_A&B** when both Syringe pumps need calibration, to remove air bubbles from the tubing.
2. Place the plate on the balance, and tare the balance.
3. Place the plate onto the carrier and run the dispense protocol created above.
4. Upon completion, carefully remove and reweigh the plate to determine the **Actual Weight**.
5. The **Expected Weight** is:
   - 16 tube = 7.680 grams
   - 32 tube = 9.216 grams

- 8 tube = 1.920 grams

6. Calculate the volume (weight) error and the backlash adjustment that needs to be made:
   - 16 tube: (7.680 - Actual Weight ÷ 24 ÷ [.0033 or .0031*]) = backlash setting
   - 32 tube: (9.216 - Actual Weight ÷ 48 ÷ [.0033 or .0031*]) = backlash setting
   - 8 tube: (1.920 - Actual Weight ÷ 12 ÷ [.0033 or .0031*]) = backlash setting
     * use .0033 for autoclavable units; use .0031 for non-autoclavable units.
     - Subtract the Actual Weight from the Expected Weight and divide by the number of columns dispensed to.
     - Divide the result by 0.0033 and round to the nearest whole number.

   Example: 7.680 - 7.550 = 0.130, 0.130/24 = 0.00542, 0.00542/0.0033 = 1.64 rounded to 2. The backlash setting needs to be adjusted by 2.

7. Adjust the backlash as necessary: select **Tools>Instrument Utilities> Syringe Dispenser** and enter the number of steps in the applicable Backlash fields in the Calibration Data group box.
8. Repeat steps 1 through 4 until the volume dispensed is within one backlash unit of being exact: **± 0.119 mL**.

Chapter 5

Qualification

This chapter provides instructions for periodically testing the instrument to verify that it meets performance specifications.

Qualification Overview **216**
Qualification Schedule **218**
Liquid Testing the EL406™ Microplate Washer Dispenser **219**
System Self-Test, Verify Information **221**
Fluid and Waste Sensor Test **222**
Ultrasonic Cleaner Test **223**
Washer Dispense Precision and Accuracy Test **224**
Vacuum Filtration Evacuation Efficiency Test **237**
Peri-pump Dispense Precision and Accuracy Tests **248**
Perform the Peri-Pump Precision and Accuracy Tests **251**
Syringe Dispenser Liquid Tests **261**
Perform the Syringe Dispense Precision & Accuracy Test **262**

Qualification Overview

Instrument verification for the EL406 involves three activities: qualification of installation and setup, qualification of routine capability, and qualification of long-term stability. These activities are called Installation Qualification **(IQ)**, Operational Qualification **(OQ)**, and Performance Qualification **(PQ)**, respectively.

Review the Qualification Schedule on page 218.

Verification testing includes:

- The **System Self Test** verifies system components, such as the vacuum, manifold, and carrier positioning. The **Checksum Test** verifies the basecode software against internal checksum values to ensure that no corruption has occurred.

Washer

- **Evacuation Efficiency:** This test measures the residual volume per well after aspiration. The lower the residuals per well, the better the evacuation efficiency of the washer.
- **Dispense Precision:** This test measures the variability of volumes dispensed from tube to tube across the manifold.
- **Buffer Switching Module:** This test measures the variability of volumes dispensed from tube to tube across the manifold when using buffer switching.
- **Vacuum Filtration Evacuation Efficiency:** When applicable, this test measures the total residual volume of the plate after evacuation to verify it is performing to specifications.

Washer Qualification Tests:

- Dispense Precision (96) on page 230
- Dispense Precision Test (192) on page 234
- Evacuation Efficiency Test (96) on page 228
- Evacuation Efficiency Test (192) on page 232
- Evacuation Efficiency Test 128 on page 235
- Buffer Switching Dispense Test on page 231
- Vacuum Filtration Evacuation Efficiency Test on page 237

Peri-pump and Syringe Dispensers

- **Dispense Precision** is a measure of the variability of volumes dispensed from tube to tube across the manifold or tip holder. The optical density of the solution in a well is proportional to the total volume of the solution in the well. When the % Coefficient of Variation (%CV) is calculated, the result is a measure of the uniformity of the

distribution of dispensed volumes across the manifold. It is the ratio, expressed in percent, of the standard deviation of the distribution of fluid volumes in the wells to the mean value of volume per well. The uniformity of distribution across the manifold improves as the %CV is lowered.

- **Dispense Accuracy** is a measure of the average volume dispensed per well. It is independent of precision. The volume per well may vary greatly over a plate, yet the accuracy may be exact because it is an average of the volumes.

Peri-pump Qualification Tests:

- Performing the Peri-Pump Precision and Accuracy Tests on page 251

Syringe Dispenser Qualification Tests:

- Perform the Syringe Dispense Precision & Accuracy Test on page 262

Qualification Schedule

The following schedule defines the factory-recommended intervals for verification tests for an instrument used two to five days a week. The schedule assumes that the EL406 is properly maintained as outlined in the Recommended Maintenance Schedule on page 167.

- **Note:** An instrument qualification package (PN 7180527) is available for purchase. The package contains thorough procedures for performing Installation Qualification, Operational Qualification and Performance Qualification (IQ-OQ-PQ) and preventative maintenance (PM). Extensive Checklists and Logbooks are included for recording results. Contact your local dealer for more information.

Tests	IQ	OQ	PQ
	Initially	Annually	Monthly
Unpacking, Installation, and Setup	✓		
Enable/Test System Sensors	✓	✓	
System Self Test and Checksum Test	✓	✓	✓
Shake Test		✓	
Ultrasonic Cleaner Test		✓	
Washer Evacuation Efficiency Test		✓	✓
Washer Dispense Precision Test		✓	✓
Vacuum Filtration Evacuation Test		✓	✓
Buffer Switching Dispense Test		✓	
Peri-pump Dispense Precision and Accuracy Test		✓	✓
Syringe Dispense Precision and Accuracy Test		✓	✓
Run Assay			✓

- **Important!** The risk factors associated with your assays may require that the Operational and Performance Qualification procedures be performed more or less frequently than shown above.

- Note for **Syringe Dispensers with Buffer Switching**: Do not use the Buffer Switching module to perform qualification tests. Connect the fluid supply directly to each pump to test dispense precision and accuracy.

Liquid Testing the EL406™ Microplate Washer Dispenser

Which Tests to Perform?

We recommend that you perform these routine tests before first use (after the IQ) and then monthly:

Washer

- **Dispense Precision and Accuracy Test.** Precision tests measure the variability of volumes dispensed from tube to tube across the manifold and Accuracy tests measure the average volume dispensed per well.
- **Evacuation Efficiency Test.** Measures the residual volume per well after the aspiration aspect of plate washing. The lower the residuals per well, the better the evacuation efficiency of the washer.

If your washer is equipped with Buffer Switching ("B" models), we recommend performing this additional test annually (starting one year after installation):

- **Annual Buffer Switching Test.** Measures the variability of volumes dispensed from tube to tube across the manifold, for each valve, when the washer is equipped with buffer switching.

Perform the tests applicable to the installed manifold type. And, for instruments with buffer switching and vacuum filtration, perform the applicable tests:

If you have:	Run Liquid Test(s):
96-Tube Manifold	Dispense Precision and Accuracy Test (96)
	Evacuation Efficiency Test (96)
192-Tube Manifold	Dispense Precision and Accuracy Test (192)
	Evacuation Efficiency Test (192)
Buffer Switching	Buffer Switching Dispense Test (Annual OQ only)
128-Tube Manifold	Evacuation Efficiency Test (128)
Vacuum Filtration Module	Vacuum Filtration Evacuation Efficiency (for either 96- or 384-well plates)

Peri-pump Dispenser

- **Dispense Precision and Accuracy Test.** Perform two tests, one at the volume that matches the cassette type and another that best represents the cassette type and dispense volume most common to your applications.

Syringe Dispenser

- **Dispense Precision and Accuracy Test.** Perform two tests for each syringe using the tests that best represent the plate type and dispense volume most common to your applications.

If you have:	Run Liquid Test(s)
8-tube manifolds (1 unit)	Test 1 and Test 2
16-tube manifolds	Test 1 and Test 2
32-tube manifolds	Test 3 (twice)

Important Recommendations for All Liquid Tests

Test Solutions

- Using pure deionized water in place of the test solutions is *not* recommended and will likely result in the failure to meet specifications.
- Prepare the solutions the day before you plan to run the tests. This will allow any foam caused by the agitation of solutions containing Tween® 20 to settle.
- BioTek determined the pass/fail specifications for the instrument tests using the recommended test solutions. You may use your own buffer solution instead, but if any tests fail using your own buffer, retry the tests using the recommended solutions.

Plate Reading

- If you are using one of BioTek's keypad-based readers, such as the ELx800 or ELx808, ensure that the reader is **not** running in **Rapid mode**. To check the setting, select **UTIL → READ** and cycle through the options until READ IN RAPID MODE? appears. Set it to **NO**.
- The absorbance of blue dye solutions should be measured at 630/450 (or 405) nm. The BioTek blue dye solution part number is **7773001**.

- The final absorbance for all dye solution concentrations should be in a range between 0.700 and 1.600 OD.

Recording Test Results

- Use the Liquid Test Worksheets at the end of this section for recording data reduction results. If your tests are failing, this information will be useful for BioTek TAC to help diagnose any problems.

System Self-Test, Verify Information

Perform these steps to verify software information and run a system self-check:

Prerequisite: When controlling the instrument with the LHC, ensure that it is attached to the host computer and turned on, and then launch the LHC software.

To run the System Self-Test:

LHC	Keypad
Select **Tools>Instrument Utilities>General Settings**. Under Instrument Functions, click Perform **Self-check**.	1. Select **UTILS** at the main menu. 2. Select **TESTS**. 3. Select **SLFCHK**.

Test Results:

- If the test passes, a "passing" message appears.
- If the test fails, an error code displays. If this happens, find the error code in the EL406 Operator's Manual to determine its cause. If the problem is something you can fix, turn off the instrument, fix the problem, and then turn the instrument back on and re-run the test. If the problem is not something you can fix, contact BioTek's Technical Assistance Center.

Record Basecode

Record the software part number and version installed on the EL406.

LHC	Keypad
1. Select **Tools>Instrument Utilities** 2. Select the Software tab and from the Basecode Software Information section record the • Software Version and part number • Data Version • Checksum values UI and MC	1. Select **UTILS** 2. Select **TESTS** 3. Select **CHKSUM** 4. Reveal and record the checksum values one at a time for UI and MC. Also record the Software part number and version.

Checksum Test

The Checksum Test compares the on-board software with an internally recorded checksum value to ensure the program has not become corrupted.

To run the Checksum Test:

LHC	Keypad
1. At the LHC main view, select **Tools>Instrument Utilities>Software**. 2. Click the **Retrieve All** onboard settings link at the top of the window.	1. Select **UTILS** at the main menu. 2. Select **TESTS**. 3. Select **CHKSUM**. 4. One at time, display the **UI** and **MC** checksums.

Fluid and Waste Sensor Test

These tests simulate an error condition for the fluid and waste alert sensors.

1. Test the Fluid Supply Sensor

1. Remove the lid on the fluid supply bottle (Buffer valve A, when applicable) and lift the internal tube up and out of the fluid.
2. Run W-DAY_RINSE to bleed the fluid lines.
3. Put a plate on the carrier and run a dispense protocol, e.g. W-96_DISP_TEST.
4. Look for air moving through the tubing to the washer and wait for the alarm.

The instrument should display a fluid error, like 1500 or 1501.

2. Test the Waste Level Sensor

1. Turn the waste bottle upside down.
2. Run an aspirate protocol, e.g. W-96_EVAC_TEST.

The instrument should beep and display the expected error code, 1401.

Ultrasonic Cleaner Test

Perform this test to verify that the cleaner is operating properly.

1. Fill a supply bottle with one liter of deionized water. Buffer Switching models need two bottles connected to valves A and B.
2. Create a protocol with an AutoClean step. Set the Duration to 00:02 (2 minutes).
3. Run the protocol.
4. While the AutoClean program is running, the ultrasonic cleaner should pulse on and off approximately every ten seconds (a 50% duty cycle). While the program is running, listen for the periodic "hissing" sound that indicates the ultrasonic energy is present. Detecting the sound is all that is required to verify that the ultrasonic cleaner is operating properly. After two minutes, the washer will aspirate the fluid and the program will be complete. (Non-Buffer Switching models can press STOP when prompted for a rinse bottle and quit the program.)

Special Procedures for 1536-well Hardware:

The 128-tube aspirate manifold cannot completely evacuate the priming trough.

1. Connect a bottle of cleanser or water to the Dispense Fluid In port on the rear of the instrument to use the single priming tube in the 128-tube aspirate manifold to fill the priming trough (ultrasonic bath);
2. When AutoClean is finished, manually remove the residual liquid using a pipette, paper towel or other method.

If the ultrasonic cleaner does not operate as described above, contact BioTek for assistance.

Washer Dispense Precision and Accuracy Test

The dispense precision test measures the **variability of the volumes dispensed** from tube to tube across the wash manifold. In this test, a blue dye solution is dispensed into a microplate. The optical density of each well is measured at 630 nm and the background at 450 nm is subtracted to account for scratches on the plate or particulates in the well.

The average error percentage is calculated and the amount dispensed to each well is calculated. Acceptance is based on the **%CV** (%Coefficient of Variation), or the ratio of the standard deviation of the distribution of fluid volumes in the wells to the mean value of volume per well. The lower the %CV, the better the uniformity across the manifold.

Dispense accuracy is a measure of the average volume dispensed per well. In this test, an empty microplate is tared on a balance. A known volume is dispensed to the plate and it is weighed again. The Total Dispense Weight is determined and compared to the expected weight.

Annual Buffer Switching Test: The Dispense Accuracy test is also conducted on instruments with the external buffer switching module to ensure that each valve (A, B, C, D) is calibrated to deliver the same volume of fluid.

See: Which Tests to Perform on page 219

Predefined Test Protocols

Several EL406 protocols are shipped onboard the instrument and with the **LHC** and installed on your computer during LHC installation.

They include the protocols required to perform the Liquid Tests:

Predefined Protocols
W-DAY_RINSE
W- 96_EVAC_TEST
W- 192_EVAC_TEST
W-1536_EVAC_TEST
W- 96_DISP_TEST
W- 192_DISP_TEST

Predefined Protocols
W-96_VAC30_TEST
W-384_VAC10_TEST
Prime_All_Buffers

The files are stored in the LHC's default file location. Unless you have changed the file location:

1.  Click the **Open** button and locate the **EL406** folder.
2. Open the **Maintenance** or **QC Protocols** folder.
3. Select the required protocol.

BioTek recommends customizing these predefined protocols, as described in the topic, Customize the Predefined Protocols on page 67.

- **192-Tube manifold** users must customize the maintenance protocols. If you use both types of manifolds, make a copy of the W-DAY_RINSE protocol and change its **Instrument Settings** to correspond to the 192-tube manifold. Preserve the original protocol for use with the 96-tube manifold.

Default Parameters for the Liquid Test Protocols

Evacuation Efficiency Test Protocols

	W-96_EVAC_TEST	W-192_EVAC_ TEST	W-1536_ EVAC_TEST
Aspirate Height (Z)	28 (3.556 mm)	16 (2.03 mm)	40 (5.08 mm)
X Position	-50 (-2.29 mm)	00 (0 mm)	00 (0 mm)
Y Position	2 (0.15 mm)	00 (0 mm)	00 (0 mm)
Travel Rate	2 (3.4 mm/sec)	3 (4.0 mm/sec)	3 (4.0 mm/sec)
Delay	0	0	0
Secondary Aspirate	No	No	No

Dispense Precision and Accuracy Test Protocols

	W-96_DISP_TEST	W-192_DISP_TEST	S-1536_DIS_TEST
Buffer Valve	A	A	See Syringe dispenser tests
Dispense Volume	300 µL/well	80 µL/well	
Flow Rate	6	7	
Dispense Height (Z)	120	120	
X Position	00	00	
Y Position	00	00	
Pre-dispense	No	No	

Vacuum Filtration Test Protocols

	W-96_VAC30	W-384_VAC10
Plate Type	96	384
Volume	300 µL/well	80 µL/well
Filtration Time	30 seconds	10 seconds

Washer Qualification Test Materials

- One new microplate per test to be performed:

Microplate Type	Liquid Tests
Flat-bottom 96-well plates, Corning® Costar #3590 or equivalent	Evacuation Efficiency Test (96)
	Dispense Precision and Accuracy Test (96)
	Annual Buffer Switching Test
Flat-bottom 384-well plates, Corning Costar or equivalent	Evacuation Efficiency Test (192)
	Dispense Precision and Accuracy Test (192)
	Annual Buffer Switching Test
Standard 1536-well plates, Nunc® #264710 or equivalent	Evacuation Efficiency Test (128)

Microplate Type	Liquid Tests
• 96-well 0.45 µm filter plate, Millipore MSHVN4550 96 or equivalent • 384-well 1.2µm filter plate, Millipore MZFCN0W10 or equivalent	Vacuum Filtration Evacuation Efficiency Test (*Use the plate size most commonly used in your lab*)

- Precision balance with minimum capacity of 100 g and readability of 0.001 g resolution
- Pipettes and graduated beakers
- Microplate absorbance reader capable of dual wavelength reading at 630/450 nm
- Liquid Test Worksheets at the end of this chapter for recordingdata and results
- Deionized water

- **Test solutions:**

For this manifold:	Sol. 1	Sol. 2	Sol. 3	Sol. 4
96-Tube	1000 mL	100 mL	1200 mL	n/a
192-Tube	2000 mL	100 mL	n/a	1440 mL
128-Tube	100 mL			

▪ These volumes are sufficient for performing the dispense tests and standard and diagnostic Evacuation Efficiency tests. In most cases, enough fluid will be left over to re-run a test, if necessary.

▪ If you will be performing the annual OQ for the external Buffer Switching module, you will need several additional liters of deionized or distilled water.

Test Solutions

Solution #1: Buffer Solution		
Pipette 1 mL Tween 20® into 1 liter (1000 mL) of deionized or distilled water and mix well.	**or**	Pipette 10 mL of BioTek Wetting Agent* into 1 liter of deionized or distilled water and mix well.

* BioTek Solution #1 100X Concentrate Wetting Agent 125 mL (PN 7773002) contains 10% Tween 20 in deionized or distilled water and 0.01% Sodium Azide as a preservative.

SOLUTION #2: Residual Test Solution		
Mix 100 mL of **Solution #1** with 0.050 grams of FD&C #1 blue dye.	**or**	Mix 90 mL of **Solution #1** with 10 mL of BioTek Blue Test Dye*.

* BioTek Solution #2 10X Concentrate Blue Test Dye 125 mL (PN 7773001) contains 5 g per liter FD&C Blue #1, 0.1% Tween 20 in deionized or distilled water and 0.01% Sodium Azide as a preservative.

SOLUTION #3: Dispense Precision and Accuracy Solution for 96-Tube manifolds
Mix 1180 mL of deionized or distilled water with 20 mL of **Solution #2**.

SOLUTION #4: Dispense Precision and Accuracy Solution for 192-Tube manifolds
Mix 1420 mL of **Solution #1** with 20 mL of **Solution #2**.

Evacuation Efficiency Test (96)

- This test is designed for **96-Tube** manifolds. Find the Evacuation Efficiency Test (192) on page 232

- If you tared the balance at the start of the Dispense Precision and Accuracy Test, use the plate from that test here; skip steps 1-3.

1. Fill a supply bottle with 2 liters of deionized or distilled water. Run the Maintenance program **W-DAY_RINSE** two or three times to prime the tubing and manifold.
2. Place a new 96-well microplate on the balance and zero the balance.
3. Pipette or dispense 150 µL of **Solution #1** into each well of the microplate.
4. Place the plate on the carrier and run the **W-96_EVAC_TEST** protocol. This protocol evacuates the wells, leaving a small amount of residual fluid.
5. When the program is completed, remove the plate and weigh it immediately because evaporation will affect the results. This is the **Total Residual Weight** in grams.
6. Visually inspect the plate and note if any wells appear to have considerably more liquid than others.
7. Use the Evacuation Efficiency Test Worksheet to perform data reduction:

- Divide the Total Residual Weight by **96** to find the **Mean Residual Weight**.
- The Mean Residual Weight should be **<= 0.002 g**.

If the Mean Residual Weight is ***greater than*** 0.002 g, or if one or more wells appear to have much more liquid than the others, the washer failed the test.

Troubleshoot as follows:

If the test fails once:

- If the problem appears to be related to particular wells, clean those aspiration tubes: run AutoClean and/or remove the manifold and thoroughly clean the tubes with the stylus (**See Remove and clean the washer manifold on page 185**). When finished, retry the test.
- Failure of this test is commonly caused by improper aspiration tube placement within the wells, usually because a microplate other than the recommended Corning Costar® 96 was used. If you must use a plate *other than* the Corning Costar 96, modify the Aspirate Height, i.e., Z position, or horizontal X or Y position parameters in a copy of the **W-96_EVAC_TEST** protocol to correct this error. After making this change, retry the test using a new microplate.

If the test fails a second time: Perform the ***Evacuation Diagnostic Test***.

Evacuation Diagnostic Test

- Conduct this test if the standard Evacuation Efficiency Test fails **twice**. This test will confirm which aspirate tube(s) may be clogged, or if the plate's alignment or position is the problem.

1. If you have not already done so, repeat steps 2 through 7 of the standard Evacuation Efficiency test, using **Solution #2** for the dispense fluid. Be sure to recalculate the **Mean Residual Weight**.
2. Pipette up to 300 µL of **Solution #1** into each well, on top of the residual solution.
3. Shake the plate to achieve uniform distribution of the remaining dye in each well.
4. Read the plate in an optical reader (blank on air), using the dual-wavelength method (630 nm - 450 nm), then print or export the results.
5. Use the ***Evacuation Efficiency Test Worksheet*** to perform data reduction:
   - Calculate the sum of the OD values for all wells, then divide by **96** to determine the **Mean OD** for the plate.
   - Divide the Mean OD by the **Mean Residual Weight** (from step 1), to find the **Residual Factor**.
   - For each well, divide its OD value by the Residual Factor to find its **Residual Weight**.

   Each well's Residual Weight should be **<= 0.002 g**.

If one or more wells have a Residual Weight *greater than* 0.002 g, review the data to determine which well, or wells, is causing the problem.

- If the problem appears to be related to particular wells, clean the associated aspiration tubes: run **AutoClean** and/or remove the manifold and thoroughly clean the tubes with the stylus. (**See Remove and clean the washer manifold on page 185**.) When finished, retry the test.
- If the problem appears to be related to a particular region, edge, or corner of the plate, review the alignment and flatness of the plate on the carrier.

Please do not adjust the carrier adjustment screws! Contact BioTek if you suspect an alignment problem, contact BioTek TAC.

- For additional suggestions, **See Troubleshooting on page 272** .
- If the test continues to fail, contact BioTek Instruments.

Dispense Precision and Accuracy Test (96)

- This test is designed for **96-Tube** manifolds. Find the Dispense Precision Test (192) on page 234.
- Find a list of the supplies you will need at Materials on page 226.
- **Save the plate!** When this test is complete, used the filled plate to perform the Evacuation Efficiency Test.

1. Fill a supply bottle with 2 liters of deionized or distilled water; Buffer Switching units use buffer valve A. Run the **W-DAY_RINSE** protocol two or three times to prime the fluid lines and manifold.
2. Fill a supply bottle with 1200 mL of **Solution #3**; Buffer Switching units use buffer valve A.
3. Run **W-DAY_RINSE** again to prime the washer with the solution.
4. Place a new 96-well microplate on the balance and zero the balance.
5. Place the plate on the carrier and run the **W-96_DISP_TEST**. This protocol dispenses 300 µL of solution to each well of the plate. It does not evacuate the solution.
6. When the protocol is completed, carefully remove the plate. Place the plate on the balance and record the **Total Dispense Weight**.
7. Read the plate in an optical reader (blank on air), using the dual-wavelength

method (630 nm - 450 nm), then print or export the results.

8. Use the ***Dispense Precision Test Worksheet*** to perform data reduction:

Tip: If you have a spreadsheet software program, enter/export all 96 values into a spreadsheet and apply your program's Standard Deviation function (e.g., Microsoft® Excel's STDEV).

   a. Calculate the **Standard Deviation**.

   b. Calculate the sum of the OD values for all 96 wells, then divide by **96** to determine the **Mean OD** for the plate.

   c. Calculate the **%CV**: (Standard Deviation / Mean OD) * 100.

   The %CV should be **<= 3.0**.

If the %CV is *greater than* 3.0, one or more dispense tubes may need to be cleaned. Run **AutoClean**, if available, and/or remove the manifold and use the stylus to clean the dispense tube(s) giving lower-than-average absorbance readings. When finished, re-prime the washer and retry the test.

9. When finished, prime with deionized water to flush out the dye solution.

Buffer Switching Dispense Test

This procedure tests all the buffer valves.

- The **Dispense Precision** test must pass before the test for Valves A-D can be performed.

1. Empty the waste bottle now, and then as needed throughout this procedure.
2. Fill each of the supply bottles connected to **Buffer valves A**, **B**, **C**, and **D** with three liters (3000 mL) of deionized water. Place the bottles on a surface level with the EL406, i.e., the adjacent (lab bench) area.
3. Run the Maintenance protocol **W-PRIME_ALL_BFRS** two or three times to prime the fluid lines, manifold, and the valves.

Repeat the following steps for each valve:

1. Edit the applicable dispense protocol, for example, **96_DISP_TEST**, to use the buffer valve currently being tested.
2. Place a new 96- or 384-well microplate on the balance and zero the balance.
3. Place the microplate on the carrier and run the dispense protocol.

4. When the program is finished, carefully remove the plate and weigh it. This is the **Total Dispense Weight** in grams.

The Total Dispense Weight should be:

- **28.8 grams ± 10%** (between 25.92 g and 31.68 g) for the 96-Tube test.
- **30.72 grams ± 10%** (between 27.65 g and 33.79 g) for 192-Tube test.
- If the weight falls above this range, the valve may be defective. Contact BioTek.
- If the weight falls below this range, the valve may be contaminated with fungi or proteins and must be cleaned using an appropriate enzyme, alcohol, or a diluted bleach solution, depending on the contaminant. **See Removing Protein Residuals and Fungi Growth on page 175** in the Maintenance section for suggestions. After cleaning the valve and tubing, retry the test. If the test continues to fail, contact BioTek.

5. Record the results in the applicable worksheet.

Evacuation Efficiency Test (192)

- This test is designed for the **192-Tube** manifold. Find the Evacuation Efficiency Test (96) on page 228.

- If you tared the balance at the start of the Dispense Precision and Accuracy Test, use the plate from that test here; skip steps 1-3.

- **Important**: You must edit the **W-DAY_RINSE** protocol to function with the 192-Tube manifold or create your own priming protocol.

1. Fill a supply bottle with 2 liters of deionized or distilled water. Run the **W-DAY_RINSE** protocol two or three times to prime the fluid lines and manifold.
2. Place a new 384-well microplate on the balance and zero the balance.
3. Pipette or dispense 80 µL of **Solution #1** into each well of the microplate.
4. Place the plate on the carrier and run the **W-192_EVAC_TEST**. This protocol evacuates all of the wells, leaving a small amount of residual fluid.
5. When the program is completed, remove the plate and weigh it immediately because evaporation will affect the results. This is the **Total Residual Weight** in grams.
6. Visually inspect the plate and note if any wells appear to have considerably more liquid in them than others.

7. Use the ***Evacuation Efficiency Test Worksheet*** to perform initial data reduction:
    1. Divide the Total Residual Weight by **384** to find the **Mean Residual Weight**.
    2. The Mean Residual Weight should be **<= 0.002 g**.

    If the Mean Residual Weight is *greater than* 0.002 g, or if one or more wells appear to have much more liquid than the others, the washer failed the test.

Troubleshoot as follows:

If the test fails once:

- If the problem appears to be related to particular wells, clean those aspiration tubes: run AutoClean and/or remove the manifold and thoroughly clean the tubes with the stylus (**See Remove and clean the washer manifold on page 185**). When finished, retry the test.
- Failure of this test is commonly caused by improper aspiration tube placement within the wells, usually because a microplate other than the recommended Corning Costar® 384 was used. If you must use a plate *other than* the Corning Costar 384, modify the Aspirate Height, i.e., Z position, or horizontal X or Y position parameters in a copy of the **W-192_EVAC_TEST** protocol to correct this error. After making this change, retry the test using a new microplate.

If the test fails a second time: Perform the ***Evacuation Diagnostic Test (192)***.

Evacuation Diagnostic Test (192)

- Conduct this test if the standard Evacuation Efficiency Test fails **twice**. This test will confirm which aspirate tube(s) may be clogged, or if the plate's alignment or position is the problem.

1. If you have not already done so, repeat steps 2 through 7 of the standard Evacuation Efficiency test, using **Solution #2** for the dispense fluid. Be sure to recalculate the **Mean Residual Weight**.
2. Pipette up to 80 µL of **Solution #1** into each well, on top of the residual solution.
3. Shake the plate to achieve uniform distribution of the remaining dye in each well.
4. Read the plate in an optical reader (blank on air), using the dual-wavelength method (630 nm - 450 nm), then print or export the results.
5. Using the ***Evacuation Efficiency Test Worksheet***, perform data reduction:

- Calculate the sum of the OD values for all wells, then divide by **384** to determine the **Mean OD** for the plate.
- Divide the Mean OD by the **Mean Residual Weight** (from step 1), to find the **Residual Factor**.
- For each well, divide its OD value by the Residual Factor to find its **Residual Weight**.

Each well's Residual Weight should be **<= 0.002 g**.

If one or more wells have a Residual Weight *greater than* 0.002 g, review the data to determine which well, or wells, is causing the problem.

- If the problem appears to be related to particular wells, clean the associated aspiration tubes: run **AutoClean** and/or remove the manifold and thoroughly clean the tubes with the stylus. (**See Remove and clean the washer manifold on page 185**.) When finished, retry the test.
- If the problem appears to be related to a particular region, edge, or corner of the plate, review the alignment and flatness of the plate on the carrier.

Please do not adjust the carrier adjustment screws! Contact BioTek if you suspect an alignment problem, contact BioTek TAC.

- For additional suggestions, **See Troubleshooting on page 272** .
- If the test continues to fail, contact BioTek Instruments.

Dispense Precision and Accuracy Test (192)

- This test is designed for the **192-Tube** manifold. Find the Dispense Precision (96) on page 230.
- Find a list of the supplies you will need at Materials on page 226.
- **Save the plate!** When this test is complete, used the filled plate to perform the Evacuation Efficiency Test.

1. Fill a supply bottle with 2 liters of deionized or distilled water; Buffer Switching units use buffer valve A. Run **W-DAY_RINSE** two or three times to flush the fluid lines and manifold.
2. Fill a supply bottle with 1440 mL of **Solution #4**; Buffer Switching units use buffer valve A.
3. Run **W-DAY_RINSE** again to prime the washer with the solution.
4. Place a new 384-well microplate on the balance and zero the balance.

5. Place the plate on the carrier and run the **W-192_DISP_TEST** protocol. This protocol dispenses 80 µL of solution to each well of the plate. It does not evacuate the solution.
6. When the program is finished, carefully remove the plate. Place the place on the balance and record the **Total Dispense Weight**.
7. Read the plate in an optical reader (blank on air), using the dual-wavelength method (630 nm - 450 nm), then print or export the results.
8. Use the ***Dispense Precision Test Worksheet***, perform data reduction:

> ***Tip:*** If you have a spreadsheet software program, enter/export all 384 values into a spreadsheet and apply your program's Standard Deviation function (e.g., Microsoft Excel's STDEV).

   a. Calculate the **Standard Deviation**.
   b. Calculate the sum of the OD values for all 384 wells, then divide by 384 to determine the **Mean OD** for the plate.
   c. Calculate the **%CV**: (Standard Deviation / Mean OD) * 100.

   The %CV should be **<= 4.0**.

   If the %CV is *greater than* 4.0, one or more dispense tubes may need to be cleaned. Run **AutoClean** and/or remove the manifold and use the stylus to clean the dispense tube(s) giving lower-than-average absorbance readings. When finished, re-prime and retry the test.
9. When finished, prime with deionized water to flush out the dye solution.

Evacuation Efficiency Test (128)

Begin this test by dispensing the test solution with Syringe A or B to a 1536-well plate using one of the predefined QC protocols provided:

SA-1536_DISP_TST	Dispense precision test protocol for 32-tube Syringe A manifold.
SB-1536_DISP_TST	Dispense precision test protocol for 32-tube Syringe B manifold.

1. Fill the Syringe dispenser's supply bottle with 100 mL of Solution 1 (see page page 261) and **PRIME** the tubing and manifold.
2. Place a new 1536-well microplate on the balance and zero the balance.
3. **RUN** the appropriate predefined protocol (listed above) to dispense 6 µL to 512 wells of the plate.

4. After performing SA-1536_DIS_TST or SB-1536_DISP_TST, run the **W-1536_EVAC_ TEST**. This protocol evacuates all of the wells, leaving a small amount of residual fluid.
5. When the program is completed, remove the plate and weigh it immediately because evaporation will affect the results. This is the **Total Residual Weight**, in grams.
6. Visually inspect the plate and note if any wells appear to have considerably more liquid in them than others.
7. Using the **Evacuation Efficiency Test Worksheet**, perform initial data reduction:
   a. Divide the Total Residual Weight by **512** to find the **Mean Residual Weight**.
   b. The Mean Residual Weight should be **<= 0.0001 g**.

      If the Mean Residual Weight is *greater than* 0.0001 g, or if one or more wells appear to have much more liquid than the others, the washer failed the test.

If the test fails, **Troubleshoot as follows:**

- If the problem appears to be related to particular wells, clean those aspiration tubes: run **AutoClean**, and/or remove the manifold and thoroughly clean the tubes with the stylus (**See Remove and clean the washer manifold on page 185**.) When finished, retry the test.
- Failure of this test is commonly caused by improper aspiration tube positioning, usually because a microplate other than the recommended Nunc® #264710 was used. If you prefer to use a different plate, modify the Aspirate Position, i.e., Z-axis to fix the height, or X- or Y-axes parameters in a copy of the W-1536_EVAC_TEST protocol. After making needed changes, retry the test using a new microplate.
- For additional suggestions, **See Troubleshooting on page 272** .
- If the test continues to fail, contact BioTek Instruments.

Vacuum Filtration Evacuation Efficiency Test

This test is designed for the EL406 *with the Vacuum Filtration accessory. Install the vacuum filtration plate carrier to perform the test. Make sure the instrument setting for* ***Plate Carrier*** *is set correctly. Use the plate size most commonly used in your lab.*

- Perform this test during the initial and annual **OQ** and the **monthly PQ**.

1. Fill a supply bottle with 2 L (two liters) of **deionized water**. Run **W-DAY_RINSE** to prime the fluid lines and manifold.
2. Place a new microplate on the balance and zero the balance:
   - 96-well 0.45 µm filter plate: Millipore MSHVN4550 96 is recommended.
   - 384-well 1.2µm filter plate: Millipore MZFCN0W10 is recommended.
3. Run the predefined protocol to first dispense deionized or distilled water into each well of the microplate and then evacuates the wells:

Plate	Predefined Protocol	Volume	Vacuum Time
96-well	W-96_VAC30_TEST	300 µL/well	30 seconds
384-well	W-384_VAC10_TEST	80 µL/well	10 seconds

4. When the protocol is finished, remove the plate, and blot the bottom of the plate on a paper towel to remove any droplets.
5. Weigh the plate immediately. This is the **Total Residual Weight**, in grams.
6. Visually inspect the plate and note if any wells appear to have considerably more liquid in them than others.
7. Use the "Vacuum Filtration Evacuation Efficiency Test" worksheet at the end of this chapter to record the results:

Residual Weight should be:

- **<= 1.2 grams** for a 96-well plate;
- **<= 4.0 grams** for a 384-well plate.

- While BioTek recommends using the Millipore filter-bottom plate listed above in this test, you may substitute other manufacturers' filter-bottom plates that you are familiar with. However, using a substitute plate **may vary your test results.**

If the Residual Weight is greater than specified, the washer failed the test. Make sure a tight seal was maintained between the filter plate and plate carrier:

- A new, defect-free filter plate was used.
- The vacuum filtration plate carrier's gasket is clean and has not been damaged by previous use or mishandling.

Correct these conditions and rerun the test or contact BioTek TAC for assistance.

Dispense Precision Test Worksheet

96-Tube Manifold

Serial Number:	

Calculations	
Standard Deviation: (calculate using spreadsheet program)	
Mean OD: (sum of all wells ÷ number of wells)	
% Coefficient of Variation: ((Standard Deviation ÷ Mean OD) x 100)	
% CV <= 3.0?	❑ Pass ❑ Fail

Date:	
Test Performed By:	

Evacuation Efficiency Test Worksheet

96-Tube Manifold

Serial Number:	

Standard Test	
Total Residual Weight:	g
Verification that wells are consistent in appearance:	❑ Pass ❑ Fail
Mean Residual Weight (Total Residual Weight ÷ 96):	g
Mean Residual Weight <= 0.002 g?	❑ Pass ❑ Fail

Evacuation Diagnostics Test (check here ❑ if not performed)	
Mean OD for the plate (Sum of all wells ÷ 96):	
Residual Factor (Mean OD ÷ Mean Residual Weight):	
Calculate the Residual Weight for each well: well OD ÷ Residual Factor	
Every Residual Weight per well <= 0.002 g?	❑ Yes ❑ No

Date:	
Test Performed By:	

Dispense Precision Test Worksheet

96-Tube Manifold

Annual Buffer Switching Test

Serial Number:	

Calculations for Valve A	
Standard Deviation:	
Mean OD (Sum of all wells ÷ Number of wells):	
% CV (Standard Deviation ÷ Mean OD x 100):	
% CV <= 3.0?	❑ Pass ❑ Fail

Calculations for Valves A-D (Annual OQ only, check here ❑ if not performed)		
	Total Dispense Weight	28.8 g ±10%? (25.92 g - 31.68 g)
Valve A	grams	❑ Pass ❑ Fail
Valve B	grams	❑ Pass ❑ Fail
Valve C	grams	❑ Pass ❑ Fail
Valve D	grams	❑ Pass ❑ Fail

Date:	
Test Performed By:	

Dispense Precision Test Worksheet

192-Tube Manifold

Serial Number:	

Calculations	
Standard Deviation: (calculate using spreadsheet program)	
Mean OD: (sum of all wells ÷ number of wells)	
% Coefficient of Variation: ((Standard Deviation ÷ Mean OD) x 100)	
% CV <= 4.0?	❑ Pass ❑ Fail

Date:	
Test Performed By:	

Evacuation Efficiency Test Worksheet

192-Tube Manifold

Serial Number:	

Standard Test	
Total Residual Weight:	g
Verification that wells are consistent in appearance:	❑ Pass ❑ Fail
Mean Residual Weight (Total Residual Weight ÷ 384):	g
Mean Residual Weight <= 0.002 g?	❑ Pass ❑ Fail

Evacuation Diagnostics Test (check here ❑ if not performed)	
Mean OD for the plate (sum of all wells ÷ 384):	
Residual Factor (Mean OD ÷ Mean Residual Weight):	
Calculate the Residual Weight for each well: well OD ÷ Residual Factor	
Every Residual Weight per well <= 0.002 g?	❑ Yes ❑ No

Date:	
Test Performed By:	

Dispense Precision Test Worksheet

192-Tube Manifold

Annual Buffer Switching Test

Serial Number:	

Calculations for Valve A	
Standard Deviation:	
Mean OD (Sum of all wells ÷ Number of wells):	
% CV (Standard Deviation ÷ Mean OD x 100):	
% CV <= 4.0?	❑ Pass ❑ Fail

Calculations for Valves A-D (Annual OQ only, check here ❑ if not performed)		
	Total Dispense Weight	30.72 g ± 10%? (27.65 g – 33.79 g)
Valve A	grams	❑ Pass ❑ Fail
Valve B	grams	❑ Pass ❑ Fail
Valve C	grams	❑ Pass ❑ Fail
Valve D	grams	❑ Pass ❑ Fail

Date:	
Test Performed By:	

Evacuation Efficiency Test Worksheet

128-Tube Aspirate Manifold

Serial Number:	

Standard Test		
Total Residual Weight:		g
Verification that wells are consistent in appearance:	❑ Pass	❑ Fail
Mean Residual Weight (Total Residual Weight ÷ 512):		g
Mean Residual Weight <= 0.0001 g?	❑ Pass	❑ Fail

Date:	
Test Performed By:	

Vacuum Filtration Evacuation Efficiency Test

96-Well Filter Plates

Serial Number:	

Test Results	
Verification that wells are consistent in appearance:	☐ Pass ☐ Fail
Residual Weight:	g
Residual Weight: <= 1.2 g	☐ Pass ☐ Fail

Date:	
Performed By:	
If required, Reviewed/Approved By:	

Vacuum Filtration Evacuation Efficiency Test

384-Well Filter Plates

Serial Number:	

Test Results	
Verification that wells are consistent in appearance:	☐ Pass ☐ Fail
Residual Weight:	grams
Residual Weight: <= 4.0 g	☐ Pass ☐ Fail

Date:	
Performed By:	
If required, Reviewed/Approved By:	

Peri-pump Dispense Precision and Accuracy Tests

Dispense Precision and Accuracy Specifications

Cassette	Precision	Accuracy
1 µL	10%CV @ 1 µL per well	± 10% @ 1 µL per well
	5%CV @ 2 µL per well*	± 5% @ 2 µL per well*
	10%CV @ 0.5 µL per well	n/a
5 µL	5%CV @ 5 µL per well	± 4% @ 5 µL per well
	2.5%CV @ 10 µL per well*	± 2% @ 10 µL per well*
10 µL	4%CV @ 10 µL per well	± 4% @ 10 µL per well
	2%CV @ 20 µL per well*	± 2% @ 20 µL per well*
* These specifications are for these dispense volumes and higher.		

- **Note**: For IQ/PQ/OQ purposes you can add 1.0% additional tolerance to the Precision %CV to accommodate various test solutions, off-peak wavelengths, reader errors, and pipette errors.

Peri-pump Precision and Accuracy Testing Methodology

Tare an empty plate on a balance. Use the Peri-pump to dispense a quantity of fluid with a known dye concentration to the wells. Weigh the plate to obtain the weight of the fluid dispensed. Pipette deionized water on top of the dye to bring the wells up to a more optically measurable volume. Read the wells in a microplate reader and determine the percentage Coefficient of Variance (%CV) among all wells, and the accuracy of the volume dispensed in each well (% Accuracy Error).

BioTek recommends performing two tests, one at the volume that matches the cassette type and another that best represents the cassette type and dispense volume most common to your applications:

Tests – Solutions	Cassette Types			
	1 µL	1 µL 1536	5 µL	10 µL
1 µL	√	√		
5 µL			√	

Tests – Solutions	Cassette Types			
	1 µL	1 µL 1536	5 µL	10 µL
10 µL	√			√
50 µL			√	
100 µL				√
1536		√		

- **1 µL Test**: Confirms the performance of the 1 µL cassettes when dispensing a single aliquot (1/4 turn of pump) into each well of the plate. It dispenses 1 µL into each well using the **1 µL Solution**, and requires an additional 150 µL of deionized or distilled water to raise the fluid level for optimal reading.

  - A single aliquot for a cassette type is the smallest volume unit recommended for it. 1 µL for the 1 µL cassette, 5 µL for the 5 µL cassette, and 10 µL for the 10 µL cassette (except that later model instruments can dispense 0.5 µL/well using a 1 µL cassette).

- **5 µL Test**: Confirms the performance of the 5 µL cassettes when dispensing a single aliquot (1/4 turn of pump) into each well of the plate. It dispenses 5 µL into each well using the **5 µL Solution**, and requires an additional 150 µL of deionized or distilled water to raise the fluid level for optimal reading.
- **10 µL Test**: Confirms the performance of the 1 µL cassettes when dispensing 10 aliquots (2 1/2 turns of pump) and the 10 µL cassettes when dispensing a single aliquot (1/4 turn of the pump) into each well of the plate. It dispenses 10 µL into each well using the **10 µL Solution**, and requires an additional 100 µL of deionized or distilled water to raise the fluid level for optimal reading.
- **50 µL Test**: Confirms the performance of the 5 µL cassettes when dispensing 10 aliquots (2 1/2 turns of pump) into each well of the plate. It dispenses 50 µL into each well using the **50 µL solution**, and requires an additional 100 µL of deionized or distilled water to raise the fluid level for optimal reading.
- **100 µL Test**: Confirms the performance of the 10 µL cassettes when dispensing 10 aliquots (2 1/2 turns of pump) into each well of the plate. It dispenses 100 µL into each well using the solution called **100 µL solution**, and requires an additional 50 µL of deionized or distilled water to raise the fluid level for optimal reading.
- **1536 Test**: Confirms the alignment of the tips; that the cassette is firing straight into the wells. Dispenses 6 µL into columns 2, 4, 19-30, 45, 47 of a 1536-well plate using the "1536 solution." Also requires performing the 1 µL Test described above.

Peri-pump Dispenser Test Materials

- 96-well plates: Corning® Costar #3590 or equivalent
- 1536-well plates: Nunc #264710

- Precision balance with readability of 0.0001 g resolution is preferable, 0.001 g resolution is acceptable, and capacity of 100 g minimum
- Pipettes and graduated beakers
- Microplate absorbance reader capable of dual wavelength reading at 630 and 450 (or 405) nm
- BioTek blue dye solution, PN 7773001, or equivalent to create the Peri-pump Dispense Precision and Accuracy Test Solutions below.

▪ **See Important Recommendations for All Liquid Tests on page 220**.

Peri-pump Dispenser Precision and Accuracy Test Solutions

Unique concentrations of the test fluid are described here, each one corresponds to a specific dispense volume. Prepare the solutions you will need to validate the cassette types and dispense volumes used most commonly in your applications.

▪ The 5 µL Solution is used to make the higher volume test solutions.

1 µL Solution

Using BioTek's 10X concentrated blue dye solution (PN 7773001), mix 5 mL of deionized or distilled water with 8 mL of the blue dye solution.

5 µL Solution

Using BioTek's 10X concentrated blue dye solution (PN 7773001), mix 100 mL of deionized or distilled water with 10 mL of the blue dye solution.

10 µL Solution

Mix 25 mL of DI or dH2O water with 20 mL of the **5 µL Solution** (described above).

50 µL Solution

Mix 45 mL of DI or dH2O water with 5 mL of the **5 µL Solution**.

100 µL Solution

Mix 40 mL of DI or dH2O water with 2 mL of the **5 µL Solution**.

1536 Solution

Mix 5 mL of 70% isopropyl alcohol with 3 mL of the **5 µL Solution** and 35 mL of DI H2O.

Perform the Peri-Pump Precision and Accuracy Tests

Prerequisite:

- Gather the required materials.
- Prepare the test solutions.
- Make a copy of the applicable worksheets. You will find them on the operator's manual CD in the Qualification chapter PDF.

Procedure:

1. Install the cassette to be tested.
2. Turn on the EL406 and make sure the cassette (CASS) type setting is correct.
3. Turn on the balance.
4. Fill a beaker or other vessel with the test solution.
5. Define a **Protocol** and save it for reuse. Set the parameters based on the desired test volume:

> ▪ A predefined protocol for the **1536 Test** is shipped with the instrument and installed on your PC when you install the LHC: **P-1536_DISP_TEST**.

- P-Dispense Add a dispense step to the protocol: **PERIP>DISP**.
- Set the **Dispense Volume** to match the Test:

Test	Volume
1 µL	1 µL
5 µL	5 µL
10 µL	10 µL
50 µL	50 µL
100 µL	100 µL
1536	6 µL

- Set the **Flow Rate** to:
  - 1 µL cassette = Medium
  - 5 µL cassette = High
  - 10 µL cassette = High
- Optionally, choose to Require the specific cassette type under test.
- LHC users: click the **Advanced options** link; retain the default Positioning settings.

- Define a Pre-dispense: set the volume to **10 µL** and the Number of Pre-dispenses (cycles) to **2**.

6. Place a clean/new microplate on the balance and tare the balance.
7. Put the Tube Organizer into the test fluid vessel and **Prime** the tubing until any large air bubbles are removed.
8. **Run** the dispense protocol.
9. Place the plate on the balance and record the **Total Dispense Weight** in the worksheet.
10. Using a calibrated hand pipette or the Peri-pump, add the specified amount of deionized water to each well to raise the fluid level to approximately 150 µL.

Test	Volume
1 µL	150 µL
5 µL	150 µL
10 µL	100 µL
50 µL	100 µL
100 µL	50 µL
1536	0 µL

11. Read the plate in an absorbance reader using the dual-wavelength method: read the plate at 630 nm and 450 nm.
12. Calculate the **Delta OD**: (630 nm - 450 nm), **Mean Absorbance**, **Standard Deviation**, and the **%CV** for the wells under test. **%CV** = (Standard Deviation ÷ Mean) * 100.
13. Print the report, obtain required signatures, and store it according to regulatory guidelines.

If one or more of your tests are failing, make sure the dispense tubes are not clogged, (follow instructions to Unclog the Dispense Tips on page 190). If that doesn't work, recalibrate the cassette and repeat the test(s). If your tests continue to fail, contact BioTek's Technical Assistance Center (TAC).

Documenting Test Results

Dispense Precision & Accuracy Test Worksheets are provided on the Operator's Manual CD in the Qualification chapter PDF. We recommend you make copies of the appropriate pages and use them to record your calculations and test results.

Alternatively, you can purchase the instrument qualification package, which contains additional tools for conducting test procedures and recording the results, including logbooks and Excel® spreadsheets.

Peri-pump Dispense Precision & Accuracy Test Worksheet
1 µL Test for 1 µL Cassette

1 µL Dispense Precision Test	
Standard Deviation (SD):	
Mean Absorbance (sum of all wells ÷ 96)	
% CV (SD ÷ Mean x 100)	%
% CV must be < 11.0%	☐ Pass ☐ Fail

1 µL Dispense Accuracy Test	
Total Dispense (Actual) Weight:	grams
Expected Weight: (mL/well x number of wells dispensed)	grams
% Accuracy Error: (Actual Weight – Expected Weight) ÷ Expected Weight x 100	%
% Accuracy Error must be < 10.0%	☐ Pass ☐ Fail
Visual verification that no well varies considerably from the others	☐ Pass ☐ Fail

Cassette Serial Number:	
Tests Performed By: **Date:**	
Reviewed/Approved By: **Date:**	

Peri-pump Dispense Precision & Accuracy Test Worksheet
10 µL Test for 1 µL Cassette

10 µL Dispense Precision Test	
Standard Deviation (SD):	
Mean Absorbance (sum of all wells ÷ 96)	
% CV (SD ÷ Mean x 100)	%
% CV must be < 6.0%	☐ Pass ☐ Fail

10 µL Dispense Accuracy Test	
Total Dispense (Actual) Weight:	grams
Expected Weight: (mL/well x number of wells dispensed)	grams
% Accuracy Error: (Actual Weight – Expected Weight) ÷ Expected Weight x 100	%
% Accuracy Error must be < 5.0%	☐ Pass ☐ Fail
Visual verification that no well varies considerably from the others	☐ Pass ☐ Fail

Cassette Serial Number:	
Tests Performed By:	
Date:	
Reviewed/Approved By:	
Date:	

Peri-pump Dispense Precision & Accuracy Test Worksheet
1536 Test

1536 Dispense Precision Test	
Standard Deviation (SD):	
Mean Absorbance (sum of all wells ÷ 384)	
% CV (SD ÷ Mean x 100)	%
% CV must be < 6.0%	☐ Pass ☐ Fail

1536 Dispense Accuracy Test	
Total Dispense (Actual) Weight:	grams
Expected Weight: (mL/well x number of wells dispensed)	grams
% Accuracy Error: (Actual Weight – Expected Weight) ÷ Expected Weight x 100	%
% Accuracy Error must be < 5.0%	☐ Pass ☐ Fail
Visual verification that no well varies considerably from the others	☐ Pass ☐ Fail

Cassette Serial Number:	
Tests Performed By:	
Date:	
Reviewed/Approved By:	
Date:	

Peri-pump Dispense Precision & Accuracy Test Worksheet
5 µL Test

5 µL Dispense Precision Test	
Standard Deviation (SD):	
Mean Absorbance (sum of all wells ÷ 96)	
% CV (SD ÷ Mean x 100)	%
% CV must be < 6.0%	☐ Pass ☐ Fail

5 µL Dispense Accuracy Test	
Total Dispense (Actual) Weight:	grams
Expected Weight: (mL/well x number of wells dispensed)	grams
% Accuracy Error: (Actual Weight – Expected Weight) ÷ Expected Weight x 100	%
% Accuracy Error must be < 4.0%	☐ Pass ☐ Fail
Visual verification that no well varies considerably from the others	☐ Pass ☐ Fail

Cassette Serial Number:	
Tests Performed By: **Date:**	
Reviewed/Approved By: **Date:**	

Peri-pump Dispense Precision & Accuracy Test Worksheet
50 µL Test for 5 µL Cassette

50 µL Dispense Precision Test	
Standard Deviation (SD):	
Mean Absorbance (sum of all wells ÷ 96)	
% CV (SD ÷ Mean x 100)	%
% CV must be < 3.50%	☐ Pass ☐ Fail

50 µL Dispense Accuracy Test	
Total Dispense (Actual) Weight:	grams
Expected Weight: (mL/well x number of wells dispensed)	grams
% Accuracy Error: (Actual Weight – Expected Weight) ÷ Expected Weight x 100	%
% Accuracy Error must be < 2.0%	☐ Pass ☐ Fail
Visual verification that no well varies considerably from the others	☐ Pass ☐ Fail

Cassette Serial Number:	
Tests Performed By:	
Date:	
Reviewed/Approved By:	
Date:	

Peri-pump Dispense Precision & Accuracy Test Worksheet
10 µL Test for 10 µL Cassette

10 µL Dispense Precision Test	
Standard Deviation (SD):	
Mean Absorbance (sum of all wells ÷ 96)	
% CV (SD ÷ Mean x 100)	%
% CV must be < 5.0%	☐ Pass ☐ Fail

10 µL Dispense Accuracy Test	
Total Dispense (Actual) Weight:	grams
Expected Weight: (mL/well x number of wells dispensed)	grams
% Accuracy Error: (Actual Weight – Expected Weight) ÷ Expected Weight x 100	%
% Accuracy Error must be < 4.0%	☐ Pass ☐ Fail
Visual verification that no well varies considerably from the others	☐ Pass ☐ Fail

Cassette Serial Number:	
Tests Performed By: **Date:**	
Reviewed/Approved By: **Date:**	

Peri-pump Dispense Precision & Accuracy Test Worksheet
100 µL Test

100 µL Dispense Precision Test	
Standard Deviation (SD):	
Mean Absorbance (sum of all wells ÷ 96)	
% CV (SD ÷ Mean x 100)	%
% CV must be < 3.0%	☐ Pass ☐ Fail

100 µL Dispense Accuracy Test	
Total Dispense (Actual) Weight:	grams
Expected Weight: (mL/well x number of wells dispensed)	grams
% Accuracy Error: (Actual Weight – Expected Weight) ÷ Expected Weight x 100	%
% Accuracy Error must be < 2.0%	☐ Pass ☐ Fail
Visual verification that no well varies considerably from the others	☐ Pass ☐ Fail

Cassette Serial Number:	
Tests Performed By: **Date:**	
Reviewed/Approved By: **Date:**	

Syringe Dispenser Liquid Tests

Dispense Precision and Accuracy Specifications

- **Important**: For **IQ/PQ/OQ** testing purposes 1.0% tolerance has been added to some of the published specifications for Precision %CV to accommodate variations in test solutions, off-peak wavelengths, reader errors, and pipette errors. % Accuracy Error is calculated for the dispense volume specified in the respective test procedure in adherence to the published specifications.

Test #	Plate Type-Manifold	Precision	Accuracy
2	**384-well plate**	< 3% CV @ 80 µL/well	< 1.25% @ 80 µL/well
1	16-tube manifold	< 6% CV @ 20 µL/well	< 5% @ 20 µL/well
2	**96-well plate**	< 3% CV @ 160 µL/well	< 1.25% @ 160 µL/well
1	8- & 16-tube manifold	< 6% CV @ 40 µL/well	< 5% @ 40 µL/well
3	**1536-well plate**	< 12% CV @ 6 µL/ well	< 5% @ 6 µL/well
	32-tube manifold		

Syringe Dispenser Test Materials

- Microplates: 384-well plates for testing the 16-tube dispensers (which can be replaced with 96-well plates if more applicable for your lab); 1536-well plates for testing the 32-tube dispensers; and 96-well plates for testing the 8-tube dispensers.
- Precision balance with readability of 0.0001 g resolution is preferable, 0.001 g resolution is acceptable, and capacity of 100 g minimum
- Pipettes and graduated beakers
- Microplate absorbance reader capable of dual wavelength reading at 630 and 405 (or 450) nm
- The test solutions: Syringe Dispenser Test Solutions on next page.

- See also **Important Recommendations for All Liquid Tests on page 220.**

Syringe Dispenser Test Solutions

20 µL Solution

- Mix 10 mL of BioTek's blue dye solution with 100 mL of deionized or distilled water to create a dilution of the concentrate. Mix 160 mL of deionized or distilled water with 20 mL of the diluted concentrate.

80 µL Solution

- Mix 120 mL of deionized or distilled water with 40 mL of the **20 µL Solution**.

1536 µL Solution

- Mix 21 mL of 70% Isopropyl Alcohol with 13 mL of the **20 µL Solution** and 150 mL of DI H2O.

Perform the Syringe Dispense Precision & Accuracy Test

Prerequisites:

- Gather the required materials: Syringe Dispenser Materials on previous page.
- Prepare the test solutions: Syringe Dispenser Test Solutions above.
- Make a copy of the applicable worksheets. You will find them on the Operator's Manual CD in the Qualification chapter PDF.

> ▪ Do not perform qualification tests while using the Buffer Switching module. Disconnect the valve box, replacing it with a direct fluid supply to the pump, if applicable.

Test Protocols

Two predefined QC protocols are provided for qualifying the 32-tube dispensers. Skip the protocol development steps in the procedure when testing these dispensers:

Onboard Name	Description
SA-1536_DISP_TEST	Dispense precision test protocol for 32-tube Syringe A manifold.
SB-1536_DISP_TEST	Dispense precision test protocol for 32-tube Syringe B manifold.

These predefined protocols dispense 6µL/well into 512 wells of a 1536-well plate (columns 2, 4, 19-30, 45, 47). For speed, efficiency and to reduce the

amount of alcohol needed, the test is designed for visual inspection of the two columns at each end of the plate, while the block of columns at the center of the plate is used for evaluating dispense precision.

Procedure:

Perform two tests for each syringe: use two plates of the applicable type, 384-well for the 16-tube manifolds (unless only 96-well plates are used in your lab), 1536-well for the 32-tube manifolds, and 96-well for the dual 8-tube manifold, and two dye solutions.

1. Prepare the syringe dispenser to be tested:
   - **Test 1:** Use the 20 µL solution
   - **Test 2:** Use the 80 µL solution
   - **Test 3:** Use the 1536 solution
2. Prime the Syringe using your preferred method: run S-DAY_RINSE_A (or B) or use the Quick Dispense menu to remove any air bubbles from the system.
3. Create and save protocols for the tests, **two** for each Syringe, A and B, as follows:

   Skip these protocol development steps for the 32-tube dispensers. And, because you will save the protocols, you only need to create them one time for the other manifolds.

   1. Define a Dispense step for each test for each manifold, A and B:

	Manifold Type	Plate Type	Volume (µL/well)
Test 1:	16-Tube	384	20
	8-Tube	96	40
Test 2:	16-Tube	384	80
	8-Tube	96	160

   2. Set Flow Rate to **2** for all tests.
   3. Save the protocol.
4. Place a clean, empty microplate on the balance and tare the balance.
5. Place the microplate on the carrier and run the protocol (created in step 3 or predefined).
6. Place the plate on the balance and record the **Total Dispense Weight**. This value will be used to calculate the % Accuracy Error.

6. **For Test 1 Only**: Use a calibrated hand pipette or the Peri-pump to dispense deionized water on top of the dye solution in the wells.
   - **384-well:** Pipette 60 µL/well (resulting in 80 µL/well)
   - **96-well:** Pipette 120 µL/well (resulting in 160 µL/well)
7. Shake the plate using the EL406, an orbital shaker or in a microplate reader for 15 seconds, or lightly tap the side of the plate with your finger to agitate the contents of the wells.
8. Read the plate in an absorbance reader using the dual-wavelength method, to reduce the influence of scratches and foreign particles that could be in the well. See the recommended wavelengths. Print or export the results.
9. Calculate and report the Mean absorbance, Standard Deviation, and the %CV for the wells under test. %CV = (Standard Deviation ÷ Mean) * 100.
10. The **% Accuracy Error** calculation is: (Actual Weight - Expected Weight) ÷ Expected Weight x 100

    Subtract the expected dispense weight (see below) from the Actual (Total) Dispense Weight (from step 5), and divide the result by the expected weight. Multiply the result by 100.

    The **Expected Dispense Weight** is the volume dispensed per well in mL multiplied by the number of wells dispensed. For example, if 40 µL is dispensed to 96 wells, the expected weight is 0.040 x 96 = 3.84 grams. We have calculated some expected dispense weights for you:

Test	# of wells	Volume µL/well	Expected Weight
Test 1:	384 wells	20	7.68 grams
	96 wells	40	3.84 grams
Test 2:	384 wells	80	30.72 grams
	96 wells	160	15.36 grams
Test 3:	512 wells (of a 1536-well plate)	6	3.012 grams

11. Analyze your test results. The following is the Pass criteria for each test:

	%CV	% Accuracy Error
Test 1:	< 6.0%	± 5.0%

	%CV	% Accuracy Error
Test 2:	< 3.0%	± 1.25%
Test 3:	< 12.0%	± 5.0%

If one or more of your tests are failing, clean the dispense tubes with the stylus, re-prime the manifold, and repeat the test(s). If your tests continue to fail, contact BioTek's Technical Assistance Center.

Documenting Test Results

Dispense Precision & Accuracy Test Worksheets are provided on the Operator's Manual CD in the Qualification chapter PDF. We recommend you make copies of the appropriate pages and use them to record your calculations and test results.

Each Worksheet records calculations and pass/fail test results for an individual test.

Syringe Dispenser Precision & Accuracy Test Worksheet

Test 1 / 96-Well Microplate/40 µL Dispense

40 µL Dispense Precision Test	
Standard Deviation (SD):	
Mean Absorbance (sum of all wells ÷ 96)	
% CV (SD ÷ Mean x 100)	%
% CV must be < 6.0%	☐ Pass ☐ Fail

40 µL Dispense Accuracy Test	
Total Dispense (Actual) Weight:	grams
Expected Weight: (mL/well x number of wells dispensed)	grams
% Accuracy Error: (Actual Weight – Expected Weight) ÷ Expected Weight x 100	%
% Accuracy Error must be < 5.0%	☐ Pass ☐ Fail
Visual verification that no well varies considerably from the others	☐ Pass ☐ Fail

Serial Number:	
Tests Performed By: **Date:**	
Reviewed/Approved By: **Date:**	

Syringe Dispenser Precision & Accuracy Test Worksheet

Test 2 / 96-Well Microplate/160 µL Dispense

160 µL Dispense Precision Test	
Standard Deviation (SD):	
Mean Absorbance (sum of all wells ÷ 96)	
% CV (SD ÷ Mean x 100)	%
% CV must be < 3.0%	☐ Pass ☐ Fail

160 µL Dispense Accuracy Test	
Total Dispense (Actual) Weight:	grams
Expected Weight: (mL/well x number of wells dispensed)	grams
% Accuracy Error: (Actual Weight – Expected Weight) ÷ Expected Weight x 100	%
% Accuracy Error must be < 1.25%	☐ Pass ☐ Fail
Visual verification that no well varies considerably from the others	☐ Pass ☐ Fail

Serial Number:	
Tests Performed By: **Date:**	
Reviewed/Approved By: **Date:**	

Syringe Dispenser Precision & Accuracy Test Worksheet

Test 1 / 384-Well Microplate / 20 µL Dispense

20 µL Dispense Precision Test	
Standard Deviation (SD):	
Mean Absorbance (sum of all wells ÷ 384)	
% CV (SD ÷ Mean x 100)	%
% CV must be < 6.0%	☐ Pass ☐ Fail

20 µL Dispense Accuracy Test	
Total Dispense (Actual) Weight:	grams
Expected Weight: (mL/well x number of wells dispensed)	grams
% Accuracy Error: (Actual Weight – Expected Weight)÷ Expected Weight x 100	%
% Accuracy Error must be < 5.0%	☐ Pass ☐ Fail
Visual verification that no well varies considerably from the others	☐ Pass ☐ Fail

Serial Number:	
Tests Performed By:	
Date:	
Reviewed/Approved By:	
Date:	

Syringe Dispenser Precision & Accuracy Test Worksheet

Test 2 / 384-Well Microplate / 80 µL Dispense

80 µL Dispense Precision Test	
Standard Deviation (SD):	
Mean Absorbance (sum of all wells ÷ 384)	
% CV (SD ÷ Mean x 100)	%
% CV must be < 3.0%	☐ Pass ☐ Fail

80 µL Dispense Accuracy Test	
Total Dispense (Actual) Weight:	grams
Expected Weight: (mL/well x number of wells dispensed)	grams
% Accuracy Error: (Actual Weight – Expected Weight) ÷ Expected Weight x 100	%
% Accuracy Error must be < 1.25%	☐ Pass ☐ Fail
Visual verification that no well varies considerably from the others	☐ Pass ☐ Fail

Serial Number:	
Tests Performed By: **Date:**	
Reviewed/Approved By: **Date:**	

Syringe Dispenser Precision & Accuracy Test Worksheet

Test 3 / 1536-Well Microplate / 6 µL Dispense

6 µL Dispense Precision Test	
Standard Deviation (SD):	
Mean Absorbance (sum of all wells ÷ 384)	
% CV (SD ÷ Mean x 100)	%
% CV must be < 12.0%	☐ Pass ☐ Fail

6 µL Dispense Accuracy Test	
Total Dispense (Actual) Weight:	grams
Expected Weight: (mL/well x number of wells dispensed)	grams
% Accuracy Error: (Actual Weight – Expected Weight) ÷ Expected Weight x 100	%
% Accuracy Error must be < 5.0%	☐ Pass ☐ Fail
Visual verification that no well varies considerably from the others	☐ Pass ☐ Fail

Serial Number:	
Tests Performed By: **Date:**	
Reviewed/Approved By: **Date:**	

Chapter 6

Troubleshooting

This chapter provides guidelines for error recovery and troubleshooting performance problems.

Troubleshooting **272**
General Operation Troubleshooting **274**

Troubleshooting

Error recovery:

First Response: Run a System Test (restart the instrument) to give the instrument an opportunity to restore its initial settings and communication capability.

LHC Users: Reboot your Computer and Instrument: When you cannot run a system test, e.g. LHC is not responding, or when running a system test doesn't resolve the issue, turn off your computer and EL406, check all the cabling, i.e. make sure your serial or USB cable is in good condition and is properly connected to the PC and instrument, and then, power them on. This should refresh the devices and reset communication parameters.

Error Codes

To find a specific error code:

- Software Error Codes on page 297 (6000-6100) - protocol errors
- System Error Codes on page 288 (0000-A500) - hardware errors

Most error conditions generate an error message that is displayed on the computer screen or keypad.

The most common error for new EL406 users is easily fixed:

306	Peri-pump Pump Cover is open. Close the pump cover and re-run protocol.

To run the Peri-pump, its pump cover must be closed, protecting both the pump and the operator.

401	Carrier Y motor failed positional verify

If the plate carrier is not installed correctly, this instrument error will be displayed. Make sure the back right corner of the carrier is correctly seated in the little black knob attached to the transport rail. A slit on the bottom of the carrier allows it to fit into place.

6045	Serial write error

LHC Users: A potentially common error, especially when using the Predefined Protocols, a "serial write" error, is easily fixed by correcting the COM port setting defined in the protocol.

810D	To communicate, instrument must be at main menu/Home screen.

LHC Users: Similarly, the 810D message appears when the instrument is busy, for example when AutoPrime is running. The LHC can only talk to the instrument when its main menu is displayed. Press the **Stop** button, if desired, to end the current process and reestablish communication with the LHC.

Technical Note: Only one of the two communication ports (COM port) on the instrument can be used at a time. They cannot be used simultaneously. You can use USB to connect the EL406 to the computer or the RS232 serial port to connect to a BioStack or similar robotic device. But you cannot use both ports simultaneously, i.e. make sure only one cable is plugged in at a time.

Keypad Control: When the BioStack is connected to your EL406, you are controlling both instruments using the keypad. Before connecting the EL406 to your computer to download basecode or for other reasons, you must first disconnect the BioStack from the EL406 and change the Instrument Setting for the BioStack: Press **Setup Menu> →> BIOSTK> CONF>MANUAL**.

General Operation Troubleshooting

This is a list of potential performance problems and their solutions.

Startup

Problem	Possible Cause	What To Do
Display (LCD) not on.	Power cord not plugged in.	Check power connection.
Carrier/manifold position error.	Manifold or carrier is being obstructed.	Remove obstruction.
	Motor, sensor, or electrical problem.	Turn instrument off, wait at least 15 seconds, and turn it back on. If the self-test does not pass, contact BioTek TAC.
	Misaligned carrier or manifold.	Contact BioTek TAC.
	Incorrect manifold setting.	Make sure the manifold setting matches the installed manifold. LHC: Tools>Instrument Utilities>Washer/Syringe Keypad: Setup Menu>WASH/SYRG>MAN

Microplate Scratches

Problem	Possible Cause	What To Do
Scratches on microplate bottom.	Microplate dispense or aspiration height adjustment too low.	Change the Dispense or Aspirate height, the Z-axis position.
	Microplate not properly seated or strips not level.	Reseat microplate in carrier or strips in holder. Make sure the carrier is clean. Try a different microplate or strip holder. If the problem is unresolved, the carrier may have to be realigned. Contact BioTek TAC.

Washer Problems

Vacuum Pump

Problem	Possible Cause	What To Do
Vacuum pump does not start, or shakes when turned on.	Vacuum pump is not turned on.	Flip the switch on the side of the vacuum pump to turn it on.
	Vacuum pump accessory cable not installed correctly.	Plug the vacuum pump accessory cable into the back of the instrument, Accessory Outlet.
	Too much residual vacuum force for pump.	Release the vacuum by loosening the waste bottle stopper. Reconnect and start again.
	Blown fuse in accessory outlet.	Plug the vacuum pump accessory cable into the back of the instrument, Accessory Outlet, not into a wall outlet.
		Increase vacuum dissipation delay. LHC: Tools>Instrument Utilities>Vacuum Dissipation Delay. Keypad: Setup Menu>→→VACDIS
		Replace fuse (PN 46055), Replace the Vacuum Pump Fuse on page 208
Repeated blown fuses.	Vacuum Dissipate Delay is set too low for the volume of the waste bottle.	See above. If not enough time is allowed for the vacuum to dissipate, the pump will try to start while it is under a vacuum. The pump draws excessive current and blows the fuse.
	Pump has been flooded.	Remove the head from the pump and inspect it for corrosion, crystalline buildup or liquid. Contact BioTek TAC for information on pump rebuilding kits.

Fluid Aspiration

Problem	Possible Cause	What To Do
Poor or uneven aspiration.	Insufficient or no vacuum.	Firmly seat the waste bottle covers. Ensure tubing is connected properly. Check all external tubing and in-line filter for kinks or clogs. If you are using an in-line vacuum filter, it may need to be replaced.
		With the vacuum pump on, remove the vacuum pump tubing from the back of the instrument. Put your finger

Problem	Possible Cause	What To Do
		over the port; if there is no vacuum, contact BioTek TAC.
	Clogged aspiration tubes on the washer manifold.	Remove and clean the washer manifold on page 185
		Make sure the microplate carrier is level and the waste valve is not touching the bench.
	Aspirate height adjustment too high or too low.	Change the aspiration height (Z-axis position) in the protocol.
	Vacuum pump failure.	Contact BioTek TAC.
Uneven aspiration of water buffer. Some wells left full.	No surfactant in the buffer, such as Tween® 20.	Add surfactant to the buffer. If this is not possible, continue below.
	Insufficient vacuum.	BioTek offers a high-flow pump for assays using only water for the wash fluid. Contact BioTek for more information.
	Protocol settings not optimized.	Optimize protocols to improve evacuation on page 66
	Aspiration tubes not properly positioned horizontally in wells.	If none of the tubes are bent, try adjusting the horizontal aspirate position (X-/Y-axis) in the protocol.
	Microplate not level in carrier, or strips not level in holder.	Reseat microplate in carrier or strips in holder. Make sure the carrier is clean. Try a different microplate or strip holder. If the problem is unresolved, the carrier may have to be realigned. Contact BioTek TAC.
Too much residual left in wells after aspiration.	Clogged vacuum filter.	If you are using an in-line vacuum filter (PN 49943), the filter may need to be cleaned or replaced.
	Waste bottle cover not properly sealed or fittings not properly connected.	Firmly seat the waste bottle stopper. Make sure tubing is connected properly.
	Manifold out of	Check for obstructions. If none are found, contact BioTek TAC.

Problem	Possible Cause	What To Do
	alignment or not moving freely.	
	Protocol requires optimization.	Optimize protocols to improve evacuation on page 66.
	Aspirate tubes are bent.	Contact BioTek TAC.

Fluid Delivery

Problem	Possible Cause	What To Do
Unable to dispense fluid — models without the Buffer Switching module.	Clogged fluid inlet filter.	Clean the fluid inlet filter.
	Inlet tube not connected.	Make sure all tubing is connected properly. Check all external tubing for kinks or clogs.
	Clogged valve	Create a protocol with several small primes, e.g. 10 mL, to try to unclog valve.
	Clogged dispense tubes on the washer manifold.	Remove and clean the washer manifold on page 185
	No wash or rinse fluid.	Fill bottles with appropriate fluid. Ensure bottles are clean and do not contain particles or organic material.
Unable to dispense fluid — models without the Buffer Switching module.	System not primed. Large air pockets in tubing.	Run W-DAY_RINSE multiple times.
	Insufficient suction, clogged tubing, or faulty valve.	Perform Washer Maintenance on page 181; If the problem persists, contact BioTek TAC.
Unable to dispense fluid — models with the Buffer Switching module.	System not primed. Large air pockets in tubing.	Run W-DAY_RINSE multiple times.
	External valve module not connected to washer, or supply	Check valve module cable and tubing.

Problem	Possible Cause	What To Do
	tubing set up incorrectly.	
	Solenoid valve not opening.	Ensure module cable is plugged into the Valve Control port on the instrument's back panel. If plugged in, contact BioTek TAC.
Plate overfills (floods).	Dispense height too high. The aspirate tubes are too far above the wells to prevent overflow.	Lower the dispense height (Z-axis position) in the protocol.
	Dispense flow rate too low.	Define a higher dispense Flow Rate in the protocol.
	Cell wash flow rate 1 or 2 is used with 384-well plates.	Specify a non-CW dispense Flow Rate when using 384-well plates.
	Aspiration tubes hit bottom of trough during Prime or Maintenance.	Manifold may not be properly seated or mounted. Contact BioTek TAC.
	In-line vacuum filter plugged.	Replace or remove the in-line vacuum filter.
	Loose covers on waste bottles.	Firmly tighten waste bottle covers.
	Dispense rate too fast for volume selected.	Specify slower dispense Flow Rate or lower volume.
	Faulty vacuum pump.	Contact BioTek TAC.
	Insufficient or no vacuum.	Firmly seat the waste bottle covers. Check all external tubing for kinks or clogs. When the program begins, you should be able to hear the vacuum pump turn on. If it is not turning on, contact BioTek TAC. If the vacuum pump turns on, remove the vacuum tubing from the back of the instrument and put your finger over the port. If there is no vacuum, contact BioTek TAC.
Uneven dispensing of fluid; wells not	Clogged dispense tubes on the washer manifold.	Remove and clean the washer manifold on page 185

Problem	Possible Cause	What To Do
filled.		
	Manifold or tubing not adequately primed.	Run W-DAY_RINSE once or twice.
	Dispense flow rate too low. Flow rate 1 or 2 CW is used with 384-well plates.	Define a higher dispense Flow Rate.
	Microplate aspiration height adjustment too high or too low.	Change the aspirate height (Z-axis position) in the protocol.

Fluid Leakage

Problem	Possible Cause	What To Do
Fluid leaking from manifold.	Defective seals.	Replace Washer Manifold O-rings and Seals on page 208
	Aspiration tubes only: vacuum too low.	Check waste connector tubes; make sure they are properly connected. If you are using an in-line vacuum filter, check the filter for clogging, and replace if necessary. Check seal of waste bottle covers. Check for air leaks in the waste tubing and bottles. Use a slower Aspiration Travel Rate.
	Uneven (not level) surface.	Make sure the surface the washer sits on is perfectly level.
Fluid leaking from underneath the instrument.	Defective tubing connector or inlet tubing.	Contact BioTek TAC.
	Leaking valve.	Contact BioTek TAC.
Fluid leaking from external tubing connector.	Defective connector.	Replace connector.
	Worn tubing.	Replace tubing or cut back tubing one inch (to remove worn section).
	Worn seal (inlet or vacuum fitting).	Replace filter or seal.

Microplate Carrier Movement

Problem	Possible Cause	What To Do
Aspiration tubes not entering wells correctly.	Microplate not properly seated or strips not level.	Reseat microplate in carrier or strips in holder. Make sure the carrier is clean. Try a different microplate or strip holder. If the problem is unresolved, the carrier may have to be realigned. Contact BioTek TAC.
	Aspirate tubes position is too wide for a movement.	Change the horizontal, X- or Y-axis aspirate position in the protocol.
	Aspirate tubes bent.	Contact BioTek TAC.
Loud, annoying noise during operation.	Plate carrier is rubbing against glide strip.	Thoroughly clean the microplate carrier and exterior surface as recommended in the maintenance procedure.

Washer Manifold Movement

Problem	Possible Cause	What To Do
Manifold position error.	Manifold movement is blocked.	Check orientation of microplate; A1 should be in the left rear corner of the plate carrier as you face the instrument. Check for and remove any obstructions. Ensure the manifold is installed properly.
	Incorrect manifold selected.	Make sure the washer manifold setting matches the installed manifold (96-, 192- or 128-tube). LHC: Tools>Instrument Utilities>Washer>Manifold Selection. Keypad: Setup Menu>WASH>MAN

Syringe Dispenser Troubleshooting

Startup

Problem	Possible Cause	What To Do
Syringe or manifold position error.	Syringe or manifold is being obstructed.	Remove obstruction.
	Motor, sensor, or	Turn instrument off, wait at least 15 seconds, turn

Problem	Possible Cause	What To Do
	electrical problem.	it back on and run a Quick Dispense routine, e.g. Prime. If the problem persists, contact BioTek TAC.
	Syringe piston not seated all the way to the bottom before fastening set screw.	Reinstall the syringe head.

Syringe Movement

Problem	Possible Cause	What To Do
Syringe position error.	Syringe movement is blocked.	Ensure the 26-pin high-density cable shipped with the Syringe is connected to the EL406's rear panel.
		Contact BioTek TAC.
	Syringe piston not seated all the way to the bottom before fastening set screw.	Reinstall the syringe piston and syringe head.

Fluid Delivery

Problem	Possible Cause	What To Do
Unable to dispense fluid.	Inlet tube not connected at manifold or at bottle.	Check all tubing.
	Supply tube inside the supply bottle is kinked or disconnected.	Straighten or connect supply tube. Optimize Performance on page 65
	Clogged dispense tubes on the manifold.	Remove and clean the manifold.
	Inlet tube is not connected to the bottom port of the syringe.	Connect the inlet tube to the lower port of the syringe pump.
	Outlet tube is not connected to the top port of the syringe.	Connect the outlet tube to the top port of the syringe pump.
	Check valve flow direction is incorrect.	Compare the flow direction of the check valves, **See Syringe Dispenser Check Valves on page**

Problem	Possible Cause	What To Do
		46.
	Check valves are stuck closed.	Clean or Replace the Check Valves on page 196
	No fluid.	Fill bottles with appropriate fluid.
	System not primed.	Run S-DAY_RINSE for once or twice for one or both syringes.
	Faulty syringe pump.	Contact BioTek TAC.
	Set screw not tightened on the syringe pump piston.	Reinstall the syringe head.
Plate overfills (floods).	Dispense height too high.	Change the Dispense Z-axis position (height).
	Volume too large for the vessel.	Define a smaller volume.
	Dispense rate too fast for volume selected.	Define a slower dispense rate or lower volume.
Uneven dispensing of fluid; wells not filled.	Clogged dispense tubes on the dispenser manifold.	Clean the Syringe Dispenser Manifold on page 193
	Manifold or tubing not adequately primed (air in fluid lines).	Run a Prime using 20 mL. Follow with a Dispense: 20 µL per well for 24 strips.
	Dispense flow rate too low.	Define a higher flow rate.
	Setscrew not tightened on the syringe pump piston.	Reinstall the syringe head as described in Autoclave the Syringe Head on page 196
	32-tube dispense manifolds are not dispensing accurately.	Calibrate the Backlash for Syringe Dispenser on page 212
Dripping dispense tubes.	Dispense tubing routed incorrectly.	The supply bottle tube must connect to the Syringe's bottom port.
Fluid jet is off-center or skewed from 32-	Minute particles of debris on the end of the tubes.	Brush away any particles from the

Problem	Possible Cause	What To Do
tube SB manifold.		end of the tube using a piece of silicon tubing. Silicon will not flake off and leave particles behind like other materials.

Fluid Leakage

Problem	Possible Cause	What To Do
Fluid leaking from manifold.	Defective seals.	Maintaining the Syringe Dispenser
	Check valves are leaking.	Clean or Replace the Check Valves on page 196
	Fittings to manifold are leaking.	Reconnect/reseat the fittings.
Fluid leaking from underneath the unit.	Defective syringe cup.	Contact BioTek TAC.
	Leaking syringe seal.	
	Defective syringe piston.	
Fluid leaking from external tubing connector.	Worn tubing.	Replace tubing.
	Defective connector.	Contact BioTek TAC.

Microplate Carrier Movement

Problem	Possible Cause	What To Do
Dispense tubes not entering well correctly.	Microplate not properly seated or strips not level.	Reseat microplate carrier, or the plate or strips in holder.
		Make sure the carrier is clean.
	Horizontal dispense position does not align the tubes in the wells.	Change the X-axis (horizontal) Position in the protocol.
	Dispense tube(s) bent.	Push the supplied stylus into the tube and then gently attempt to straighten the tube using your

Problem	Possible Cause	What To Do
		fingers. If it remains bent, contact BioTek TAC.
	Manifold tilted.	Check tubing for twists.
Carrier position error.	Carrier movement is blocked.	Check for/remove any obstruction.
	Dirty carrier or carrier rail.	Clean carrier and/or carrier rail.

Dispense Manifold Movement

Problem	Possible Cause	What To Do
Manifold position error.	Manifold movement is blocked.	Check the dispense height or Z-axis positioning. Allow at least 1 mm clearance above plate.
		Check for/remove any obstructions.
		Contact BioTek TAC.

Peri-pump Troubleshooting

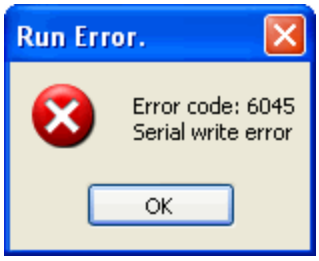
Problem	What To Do
Fluid stream missing wells	Check Tip Holder, make sure it is properly seated in the Dispense Arm. Select the correct Plate Type.
Fluid splashing out of the wells	Select the correct Plate Type. Reduce the Flow Rate. Lower the Dispense Height.
Uneven dispensing	Make sure all cassette components are properly seated in their respective positions. Tips are clogged. (See the Preventive Maintenance chapter.) Recalibrate the cassette. Replace the tubing. (On the Operator's Manual CD, in the PDF folder, find instructions for recalibrating the cassette and replacing the tubing.)
Dispenser skipping	Check/define the Plate Map.

Problem	What To Do
columns	
Tips clogging	Filter the dispense fluid to 50 microns before dispensing. Replace the tubing.
Viscous fluids sticking to tips	Vary the Flow Rate: experiment with different flow rates to determine which setting best forces fluid to break from the tip.
Cannot communicate with computer	Check the cabling. (See previous section.) Select the correct COM Port. Turn on dispenser; display Main Menu.
Foaming in the wells	Reduce the dispense step's Flow Rate.

Communication Errors

Here are some guidelines for troubleshooting communication errors between the EL406 and the computer.

6045: A potentially common error, especially when using the Predefined Protocols, a "serial write" error, is easily fixed by correcting the COM port setting.



810D: Similarly, the 810D message appears when the instrument is busy, for example when **AutoPrime** is running. The LHC can only talk to the instrument when its main menu is displayed. Press the **Stop** button on the keypad, if desired, to end the current process and return to the main menu.

Safety first

- To prevent damage to the instrument, always turn OFF the EL406 or the computer before removing or inserting a communications (serial or USB) cable.

When the computer (PC) won't communicate with the instrument:

1. **Run the system self-test**. All BioTek instruments perform a self-test when turned on. The EL406 will not communicate if it fails an internal system test. An error message will be displayed when a test fails.

2. **Make sure the serial or USB cable is in perfect condition and properly attached** to the port defined in the Instrument Settings dialog (e.g. COM 1). Review the LHC Help Topic Select Help>Help Topics and search for "About COM Ports." "About COM Ports" to learn about virtual COM ports when using a USB cable. Correct and reboot both PC and instrument. Test communication.
3. **Confirm that the serial/USB cable was obtained from BioTek**. Serial/USB cables are not universal. Contact BioTek customer service to purchase a factory tested cable. After installing a known, good cable, reboot both PC and instrument.

Appendix A

Error Codes

A listing of potential error codes and possible solutions for resolving them.

System Error Codes **288**
EL406 Software Error Codes **297**

System Error Codes

Most of these error conditions require technical expertise to correct. Error code 306 and few other exceptions to this rule are listed with remedies in the Troubleshooting section. A few other errors may be caused by an obvious obstruction to a device's movement or insufficient fluid in a supply vessel. Fix these kinds of errors and restart your instrument to give it an opportunity to clear the error code.

Contact BioTek Technical Assistance Center (TAC) for assistance.

Code	Message	What to do
100	Task was aborted	Restart instrument if this message is unexpected.
210, 220	Carrier X motor didn't find home opto sensor transition Carrier X motor didn't find autocal jig opto sensor transition	Clean the plate carrier, rails, and glide strips, using mild detergent and hot water, 70% isopropyl alcohol or ethanol. Restart the instrument. If the error occurs again, contact BioTek TAC.
211, 221	Carrier Y motor didn't find home opto sensor transition Carrier Y motor didn't find autocal jig opto sensor transition	Run self test. If error reoccurs, contact BioTek TAC.
212, 222	Dispense head motor didn't find home opto sensor transition, Dispense head motor didn't find autocal jig opto sensor transition	Run self test. If error reoccurs, contact BioTek TAC.
213, 223	Wash head motor didn't find home opto sensor transition, Wash head motor didn't find autocal jig opto sensor transition	Run self test. If error reoccurs, contact BioTek TAC.
214	Syringe A motor didn't find home opto sensor transition	Run self test. If error reoccurs, contact BioTek TAC.
215	Syringe B motor didn't find home opto sensor transition	Run self test. If error reoccurs, contact BioTek TAC.

Code	Message	What to do
216	Peri-pump motor didn't find home opto sensor transition	Run self test. If error reoccurs, contact BioTek TAC.
220	Carrier X motor didn't find autocal jig optical sensor transition.	Service Only. Contact BioTek TAC.
221	Carrier Y motor didn't find autocal jig optical sensor transition.	Service Only. Contact BioTek TAC.
223	Wash head motor didn't find autocal jig optical sensor transition.	Service Only. Contact BioTek TAC.
300	Carrier X motor interlock safety switch open	Service Only. Contact BioTek TAC.
301	Carrier Y motor interlock safety switch open	Service Only. Contact BioTek TAC.
302	Dispense head motor interlock safety switch open	Service Only. Contact BioTek TAC.
303	Wash head motor interlock safety switch open	Service Only. Contact BioTek TAC.
304	Syringe A motor interlock safety switch open	Service Only. Contact BioTek TAC.
305	Syringe B motor interlock safety switch open	Service Only. Contact BioTek TAC.
306	Peri-pump Pump Cover is open	Close the pump cover door and rerun the protocol.
400	Carrier X motor failed positional verify	Run self test. If error reoccurs, contact BioTek TAC.
401	Carrier Y motor failed positional verify	Run self test. If error reoccurs, contact BioTek TAC.
402	Dispense head motor failed positional verify	Run self test. If error reoccurs, contact BioTek TAC.
403	Wash head motor failed positional verify	Service Only. Contact BioTek TAC.
404	Syringe A motor failed positional verify	Service Only. Contact BioTek TAC.

Code	Message	What to do
405	Syringe B motor failed positional verify	Service Only. Contact BioTek TAC.
406	Peri-pump motor failed positional verify	Service Only. Contact BioTek TAC.
600-606	Specified motor currently in use	Service Only. Contact BioTek TAC.
700 - 70F	Invalid motor number specified	Service Only. Contact BioTek TAC.
900	Calibrationon failed. The measured or calculated autocal value is out of tolerance	Service Only. Contact BioTek TAC.
A00	Invalid plate type selected	The currently selected plate type is not supported with the currently installed or requested hardware. If this is not the case, contact BioTek TAC.
C01	Configuration or autocal data missing	Service Only. Contact BioTek TAC.
C02	Checksum mismatch -calculated checksum didn’t match saved checksum	Service Only. Contact BioTek TAC.
C03	Configuration parameter out of range	Service Only. Contact BioTek TAC.
1001	Bootcode powerup checksum test failed	Contact BioTek TAC.
1002	Unknown error in bootcode	Contact BioTek TAC.
1003	Bootcode page program error	Contact BioTek TAC.
1004	Bootcode block size error (not 256)	Contact BioTek TAC.
1005	Invalid processor signature (not 1280,1281,2560,2561)	Contact BioTek TAC.
1006	Bootcode memory exceeded	Contact BioTek TAC.
1007	Invalid slave port	Contact BioTek TAC.
1008	Invalid response from slave	Contact BioTek TAC.

Code	Message	What to do
1009	Invalid processor detected	Contact BioTek TAC.
1010	Checksum error downloading basecode	Contact BioTek TAC.
1250	Internal RAM test error on the UI processor	Contact BioTek TAC.
1251	Internal RAM test error on the MC processor	Contact BioTek TAC.
1260	Stack test error on the UI	Contact BioTek TAC.
1261	Stack test error on the MC	Contact BioTek TAC.
1300	Invalid syringe selection	Contact BioTek TAC.
1301	Syringe module not connected	Make sure the Syringe module is correctly connected using the new BioTek-provided serial cable.
1302	Syringe initialization error	
1303	Syringe sensor not cleared error	
1304	Invalid syringe dispense volume	See error 1308 below.
1305	Invalid syringe operation	Contact BioTek TAC.
1306	FMEA error on syringe A	Contact BioTek TAC.
1307	FMEA error on syringe B	Contact BioTek TAC.
1308	Invalid Syringe pre-dispense volume	Protocol may have been written for a different type of dispense manifold. Make sure the Instrument Settings represent the installed hardware. Modify the protocol to match.
1309	Invalid Syringe prime volume	
1310	Invalid Syringe manifold	
1350	Peri-pump (PP) invalid dispense volume	Contact BioTek TAC.
1351	PP invalid cassette type	Change the cassette type to match the protocol requirement and rerun the protocol.
1352	PP invalid pre-dispense volume	Contact BioTek TAC.
1353	Multiple required cassettes	Make sure every Peri-pump step in the protocol calls for the same cassette type. A conflict was found.

Code	Message	What to do
1354	PP pump cover (safety door) open	Close the pump cover door and rerun the protocol.
1355	PP not installed	If you are trying to run a Secondary Peri-pump, check the cabling in the back of the instrument. Otherwise Contact BioTek TAC.
1356	PP Invalid dispense position	Contact BioTek TAC.
1357	PP two dispensers are expected	Contact BioTek TAC.
1400	No vacuum pressure detected after turning on the vacuum pump	Make sure the vacuum pump is turned on, and not leaking; the waste bottle has not overflowed and its caps are seated correctly. If the pump is not running, the fuse may be blown. But, before replacing the fuse, first try to determine and remedy the cause of the failure. If the pump sounds strained or runs slowly, it may be damaged. Review other related Troubleshooting suggestions.
1401	The waste bottles should be emptied before continuing	Empty the waste bottles and rerun the protocol.
1402	The requested valve is invalid	Contact BioTek TAC.
1403	Invalid Z-axis offset value	The Magnet Adapter offset (MAGHT) is too high, the "in use" value is missing, or a more serious software problem exists. Make sure the setting is valid, and if so, contact BioTek TAC.
1404	Plate type restricted	This instrument model or the requested device does not support the selected plate type. Edit the protocol to change the plate type.
1405	Z-axis out of range	The requested travel/dispense height cannot be reached. The conflict may be caused by a combination of variables, plate type, Plate Clear or other height settings. Review the protocol parameters and instrument settings to identify a correction.

Code	Message	What to do
1407	Invalid step type	The protocol may have been created for a different instrument, it is not compatible with this instrument.
1408	Invalid plate geometry	Contact BioTek TAC.
1409	Invalid plate carrier type	Contact BioTek TAC.
1410	Incompatible hardware	Vacuum filtration requested but either the instrument is in BioStack mode, or this instrument is not configured to support it. Change the BioStack configuration, it is not compatible with the vacuum carrier, or contact BioTek TAC.
1411	Invalid plate carrier	The vacuum filtration carrier must be installed to run this kind of protocol, and the plate carrier setting must represent the currently installed carrier.
1412	Tip clearance error	Contact BioTek TAC.
1413	AutoPrime in progress	Contact BioTek TAC.
1414	AutoPrime aborted	Contact BioTek TAC.
1415	AutoPrime value out of range	Contact BioTek TAC.
1500	No buffer fluid detected at the start of a wash protocol	Fluid detection errors. Make sure the supply bottle is full and properly connected, the tubing is not kinked, blocked, etc., and the correct port/valve is selected.
1501	No buffer fluid detected before the washer dispense step	
Note:	Running very low density fluids, like alcohol, may generate some of these fluid detection errors because the fluid prohibits the float detector from operating properly. Disable the Fluid sensor (Washer Settings) while using such low density fluids, but be vigilant about monitoring fluid levels.	
1502	The buffer valve selection is invalid	Contact BioTek TAC.
1503	Dispense volume error	Contact BioTek TAC.
1504	No buffer detected flowing through the manifold during an operation	Flow detection errors. Make sure the supply bottle is full and properly connected, the tubing is not kinked, blocked, etc., and the correct port/valve is selected.
1505	No buffer detected at the end of a wash protocol	

Code	Message	What to do
1506	The requested carrier Y-axis position is out of range	Contact BioTek TAC.
1507	Internal valve transition error	Contact BioTek TAC.
1508	Pre-dispense volume error	The specified Pre-dispense volume is invalid for this plate type. Edit the protocol.
1509	Low flow 192-tube manifold error	The 192-tube manifold does not support cell wash or low-flow protocols. A wash step mismatch has occurred. Edit the protocol.
1510	Low flow 96-tube manifold error	Contact BioTek TAC.
1511	External Buffer Switching valve module is required	The current protocol is defined to use different valves of the Buffer Switching module, which is not installed. Edit the protocol.
1512	Invalid plate type	The specified plate type is not supported with the current hardware.
1513	Plate type - manifold conflict	Change the Plate Type to one supported by the manifold. Or, click the Instrument Settings link and make sure they match the physical hardware: Get settings from instrument.
1514	Ultrasound module not connected	The protocol may have been created for a different instrument, according to the instrument's basecode it is not compatible with this instrument. Contact BioTek TAC.
1515	Cell Wash hardware not installed	
1516	Vacuum filtration start or end error	Vacuum pressure is detected in the intermediate waste bottle before or after the run. Conatct BioTek TAC.
1517	Vacuum filtration sensor error	Unable to read vac. filtration sensor. This is expected if you are running with very low pressure, in this case disable the sensor. Otherwise, check for leaks, e.g. bottle cap.
1600-160C	The onboard storage space allocated for this function has been used up.	Use the LHC ”Manage Memory” control to reallocate space.
160D	Not a valid step	Contact BioTek TAC.

Code	Message	What to do
2400	Parameter limit exceeded	Contact BioTek TAC.
4000	Program locked so operation denied	Contact BioTek TAC.
4010	Program cannot be erased so delete denied	Contact BioTek TAC.
4020	Bad checksum when reading program from EEPROM	Contact BioTek TAC.
4030	Program not found	Contact BioTek TAC.
4040	Can’t save program because no space available	Contact BioTek TAC.
4050	Program run canceled by user	Restart instrument if this message is unexpected.
8100	Communications NAK	Contact BioTek TAC.
8101	Timeout while waiting for serial message data	Contact BioTek TAC.
8102	Instrument busy and unable to process message	Contact BioTek TAC.
8103	Receive buffer overflow error	Contact BioTek TAC.
8104	Checksum error	Contact BioTek TAC.
8105	Invalid structure type in byMsgStructure header field	Contact BioTek TAC.
8106	Invalid destination in byMsgDestination header field	Contact BioTek TAC.
8107	Request object received not supported by instrument	Contact BioTek TAC.
8108	Message Body size exceeds max limit	Contact BioTek TAC.
8109	Max number of requests currently running and cannot run the latest request	Contact BioTek TAC.
810A	No request running when response request issued	Contact BioTek TAC.

Code	Message	What to do
810C	Response for outstanding request not ready yet	Contact BioTek TAC.
810D	To communicate with the LHC, the instrument must be at its main menu	The LHC can only talk to the instrument when its main menu is displayed. When the instrument is busy, for example when AutoPrime is running, press the Stop button on the keypad, if desired, to end the current process and return to the main menu.
810E	One or more request parameters are not valid	Contact BioTek TAC.
810F	The command was received while the software was not ready to accept that command	Contact BioTek TAC.
A00	Invalid plate type requested	Service Only. Contact BioTek TAC.
A100 - A10F	Software device not available	Service Only. Contact BioTek TAC.
A200	Version strings for multiple microprocessors do not match	Service Only. Contact BioTek TAC.
A301	+5v logic power supply level error	Service Only. Contact BioTek TAC.
A302	+24v system/motor power supply level error	Service Only. Contact BioTek TAC.
A303	+42v Peri-pump motor power supply level error	Service Only. Contact BioTek TAC.
A305	+42v Secondary Peri-pump motor power supply level error	Service Only. Contact BioTek TAC.
A400	Malloc failed	Service Only. Contact BioTek TAC.
A500	Multiple tasks attempted to use display simultaneously	Service Only. Contact BioTek TAC.
A600	Serial EEPROM access error	Service Only. Contact BioTek TAC.
A700	Motor truncation error	Service Only. Contact BioTek TAC.

EL406 Software Error Codes

Generally, these errors are caused by protocol parameters that conflict with the instrument's onboard settings. The protocol may have been originally created for a different hardware configuration, the 192-tube wash manifold instead of the 96-tube, for example.

Quick Fix: Make sure your **Instrument Settings** accurately reflect your instrument's hardware configuration and then, modify the protocol to fix any invalid parameters. With the EL406 connected to and communicating with your computer and its main menu displayed on the keypad:

1. Click the **Settings** link in the main view.
2. In the **Instrument Settings** dialog, click the instrument link to get the settings from the instrument.
3. Modify the protocol step that generated the error message.

Error Code	Description	Help
6000	General communication error during download.	See Communication Errors on page 285
6001	COM port created by USB converter no longer active	See *Communication Port*
6002	Invalid basecode part number; instrument is not an EL406	Service Only. Contact BioTek TAC.
6003	Invalid Basecode Data Version; basecode needs to be updated	Contact BioTek to obtain latest basecode.
6004	No rows are selected for the specified plate type	Modify the protocol to select a row.
6005	Invalid row selection value (must be 0 or 1)	Contact BioTek TAC.
6006	This instrument can only process 96-well plates	The protocol may have been created for another instrument, change the plate type or select another protocol.
6007	This instrument can only process 1536-well plates	
6008	The 8-tube Syringe Manifold can only be used with 96-well plates	Mismatch between installed hardware and protocol parameters: change the plate type or correct the instrument settings to

Error Code	Description	Help
		match the currently installed hardware.
6009	The 96-tube singe wash manifold can only be used with 96-well plates	
6010	The data is invalid or out-of-range.	Service Only. Contact BioTek TAC.
6011	This step type cannot be downloaded.	Review the limitations to transferring protocols to the instrument, See the LHC Help Topic: *Transferring Protocols*.
6012	Illegal characters in protocol name	See the LHC Help topic: *Define a Protocol.*
6013	The protocol name length must be 16 characters or less.	
6014	A 1536 well plate is not supported by this instrument.	Service Only. Contact BioTek TAC.
6015	The specified volume exceeds the cassette maximum limit.	Modify the volume or change the cassette type.
6016	The volume is out-of-range.	Modify the volume or change the cassette type.
6017	Invalid flow rate.	Learn about the *Syringe Dispense Step*
6018	Invalid number of pre-dispenses.	Service Only. Contact BioTek TAC. These codes indicate an unexpected software error that cannot be fixed without BioTek support.
6019	Invalid horizontal dispense position.	
6020	Invalid dispense height.	
6021	Invalid plate clear height.	
6022	Invalid column selection value (must be 0 or 1).	
6023	Invalid protocol step type.	
6024	The Definition String contains invalid data.	
6025	Manifold conflict between protocol requirements and instrument configuration.	Change the Washer Manifold or change the Instrument Setting. See ***Appendix B*** in the operator's manual.
6026	Valve module conflict between protocol requirements and	Make sure the Buffer Switching setting matches your instrument; see

Error Code	Description	Help
	instrument configuration.	Instrument Settings.
6027	Syringe module conflict between protocol requirements and instrument configuration.	Make sure the Syringe dispenser setting matches your instrument: see Instrument Settings.
6028	Filter washer conflict between protocol requirements and instrument configuration.	Service Only. Contact BioTek TAC.
6029	Required cassette does not match installed cassette.	Change the cassette type to match the protocol requirement and rerun the protocol.
6030	Invalid cassette type was specified.	Service Only. Contact BioTek TAC.
6031	Cannot use a 96-well plate with a 192-tube manifold.	Modify the Plate Type or Change the Washer Manifold.
6032	Downloading Protocols is not supported.	Service Only. Contact BioTek TAC.
6033	This step is not supported for 1536-well plates.	Fix the plate type or the Instrument Settings. A conflict between the plate type and installed hardware devices has been detected. Change the Plate Type to one supported by the washer/dispenser. Or, click the Instrument Settings link and make sure they match the physical hardware: Get settings from instrument.
6034	The 32-tube Syringe Manifold is required for 1536-well plates.	
6035	The 16-tube Syringe Manifold is required for 96- and 384-well plates.	
6036	The 128-tube Washer Manifold is required for 1536-well plates.	
6037	The 128-tube Washer Manifold can only be used for 1536-well plates.	
6038	This step only applies to 1536-well plates.	
6039	Conflicting column selection	Fix the plate map (selected columns to dispense to). A protocol parameter may have been changed after a partial plate dispense was defined.

Error Code	Description	Help
6040	Invalid baud rate	Service Only. Contact BioTek TAC. These codes indicate an unexpected software error that cannot be fixed without BioTek support.
6041	Invalid data bits selection	
6042	Invalid stop bits selection	
6043	Invalid parity selection	
6044	Serial port error	Fix the COM port setting. Check the cabling. Click the Port link and use the drop-down menu to see all active ports. Customize the Predefined Protocols to avoid this error in future. When controlling the BioStack with the LHC, make sure the instrument's BioStack setting is **Manual**.
6045	Serial write error	
6046	Serial read error	
6047	Checksum error	Contact BioTek TAC.
6048	Serial NAK error	Make sure the COM port setting is correct and the cable is properly connected. Restart the instrument. If error reoccurs, contact BioTek TAC.
6049	Excess data, or not enough data, received.	To correct these errors: • Reset the instrument. • Check cables, plug in only one communication cable at a time: USB or serial. • Try running a different protocol. If error reoccurs, contact BioTek TAC.
6050	Invalid message header	
6051	Invalid message object	
6052	Invalid message body size	
6053	Serial message timeout	
6054	Port handle error	
6055	Read timeout value is invalid.	

Error Code	Description	Help
6056	Unauthorized to open the COM port	Make sure the COM port setting is correct and the cable is properly connected. Restart the instrument. If error reoccurs, contact BioTek TAC.
6057	Out-of-range parameter for the open port function.	
6058	Unable to open the COM port.	
6059	Unable to clear the transmission buffer.	
6060	Unable to close the port.	
6061	Port is no longer available.	
6062	Unhandled exception while transmitting message	Contact BioTek TAC
6063	The selected plate type is not allowed with this protocol step	Modify the protocol to change the plate type.
6064	The protocol specifies more Peri-pumps than are available	The protocol may have been created for a different instrument. Make sure the instrument settings match the current instrument and modify the protocol.
6065	Too few data bytes received from the instrument	Contact BioTek TAC.
6066	Ultrasonic cleaning assembly is not installed	The protocol may have been created for a different instrument. Make sure the instrument settings match the current instrument and modify the protocol.
6067	The type of Syringe pump is not compatible with the syringe manifold	Contact BioTek TAC.
6070	Invalid Syringe specified.	Service Only. Contact BioTek TAC. These codes indicate an unexpected software error that cannot be fixed without BioTek support.
6071	Invalid number of syringe prime cycles	
6072	Invalid syringe Aspirate Delay value	
6073	Invalid X-axis offset value	

Error Code	Description	Help
6074	Invalid Y-axis offset value	Service Only. Contact BioTek TAC. These codes indicate an unexpected software error that cannot be fixed without BioTek support.
6075	Invalid Z-axis offset value	
6076	Vacuum filtration not allowed with 1536-well plates.	Modify the protocol to change the plate type.
6080	Invalid Peri-pump prime duration	Service Only. Contact BioTek TAC. These codes indicate an unexpected software error that cannot be fixed without BioTek support.
6085	Invalid minutes:seconds value	
6086	Invalid hours:minutes value	
6087	'Move carrier home' is required when duration exceeds 1 minute.	Contact BioTek TAC
6088	Invalid Shake/Soak options selected	Service Only. Contact BioTek TAC. These codes indicate an unexpected software error that cannot be fixed without BioTek support.
6089	Invalid Shake Intensity selected	
6090	Invalid Washer buffer selected	
6091	Invalid Washer Aspirate Delay value	
6092	Invalid Washer Aspirate Travel Rate value	
6093	Invalid Wash Cycles value	
6094	Invalid Wash format selected	
6095	Invalid Wash Sectors selected	
6096	Wash Aspirate Delay value is required.	
6097	Syringe Dispense Volume must be an integer.	
6098	Peri-pump cannot run with the pump cover open.	Close the pump cover door and rerun the protocol.
6099	Peri-pump assembly not installed.	Physically install the Peri-pump and/or make sure the instrument settings reflect the current state.

Error Code	Description	Help
6100	This functionality requires the software to be registered.	You must register the software with BioTek. Select **Help>Register Software**.

Appendix B

Changing the Washer Manifold

Instructions for changing the washer manifold: accessory manifolds, 192-tube for processing 384-well plates and 128-tube aspirate manifold for processing 1536-well plates, replace the standard 96-tube manifold.

Changing the Wash Manifold **306**

Changing the Wash Manifold

The EL406 supports multiple types of wash manifolds. All manifolds fit onto the instrument and are removed from it in a similar manner. Accessory manifolds are shipped in special packaging. Use the shipping case to store whichever manifold is not being used.

Prerequisite:

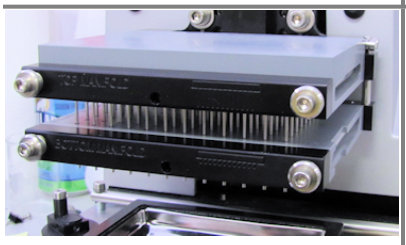
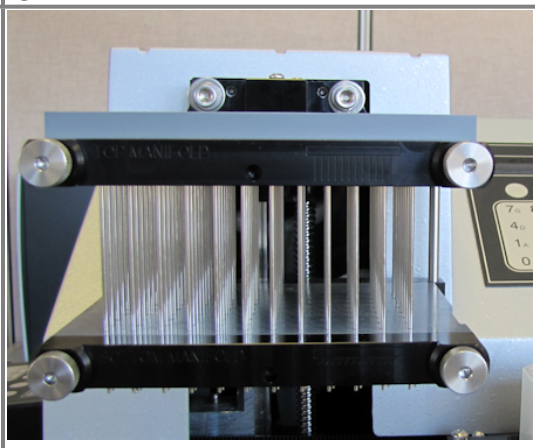
Before removing the installed manifold, run a Maintenance protocol (such as W-DAY_RINSE) or the "Long_Shutdown" procedure described in the Maintenance chapter to flush any remaining residue from the manifold tubes, if the washer has been in operation.

Tip: It is easier to clean the manifold before removing and storing it than afterwards when residuals in the tubing and fluid paths have been allowed to dry or crystallize during storage.

First, clean and dry the manifold:

- Alternatively, use the Ultrasonic Advantage™: Run AutoClean to clean the manifold.

1. Run W-DAY_RINSE one or two times with deionized water in the supply bottle.
2. Run the system "dry": Connect an empty supply bottle and run **W-DAY_RINSE**.
3. Turn off the instrument and disconnect the power cable.

Standard dual manifold	Quick release dual manifold
	
Dual 96-tube manifold	Deep-well manifold (ELx405™)

Replace the manifold:

1. Remove the mist shield.
2. **Standard dual manifold**: Using the 9/64" (3.57 mm) Allen wrench supplied with the instrument, remove the screws, washers, and springs that hold the manifold in place, and set them aside.
   Quick-release manifold: Release the thumbscrews that secure the manifolds in place.
3. Carefully remove the manifold and end plates, if applicable, holding the upper and lower manifolds together as a single unit; the 128-tube manifold is a single unit and does not have end plates.
4. Place the manifold into the shipping case for safe storage. If you ran W-DAY_ RINSE or an AutoClean protocol as instructed, ensure that the manifold is thoroughly dry before storing it.
5. Install the alternate manifold and end plates, if applicable, carefully holding the upper and lower manifolds together as a single unit, and making sure that the two O-rings do not fall out of their grooves during installation. Do not over-tighten the manifold mounting screws.

> ▪ **Important!** When reinstalling the manifold, only tighten the screw-washer-spring assembly that holds it in place until you feel the mechanical stop. Tightening past this point will damage the instrument and will **void your warranty.**

Reinstall the mist shield:

1. Align the mist shield with the washer so it rests on top of the two posts and the two thumbscrew holes in the shield are lined up with the two holes in the base of the washer.
2. Insert the two thumbscrews and finger-tighten only.

Reconfigure the instrument:

Update the washer manifold setting:

1. Using the LHC: select **Tools> Instrument Utilities> Washer**
   Using the Keypad: press **Setup Menu>WASH>MAN**
2. Choose the option that represents the installed manifold.
3. LHC users: click **Send** to send this setting to the EL406.

> ▪ **Important!** The correct manifold must be chosen as the **Manifold Selection** before operating the washer. Failure to set the manifold type before operation

may damage the manifold and void your warranty.

Storing the Unused Manifolds

96- and 192-Tube and Deep-well (ELx405 only) Boxes

- Separate the dual manifolds to store them. Put the bottom manifold in the left recess and the top manifold in the right recess.

128-Tube Box

- Hold the dual manifolds together and gently lower them into the single recess of the 128-tube manifold box. Then place the end plates, one at a time, into the same recess, on the left side of the manifolds. All components will fit snugly into place.

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