MultiFlo™

Operator's Manual

BioTek®

MultiFlo™ Microplate Dispenser Operator's Manual

BioTek Instruments, Inc.
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Part Number 7211000
Revision B

Notices

BioTek® Instruments, Inc.

Highland Park, P.O. Box 998

Winooski, Vermont 05404-0998 USA

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Contents

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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	10/2010	First Issue
B	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.

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 MultiFlo. 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 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.

Intended Use Statement

- The MultiFlo™ Microplate 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.

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! 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: 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 61000-4-11, 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."

EN 61010-2-081, "Particular requirements for automatic and semi-automatic laboratory equipment for analysis and other purposes."

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.

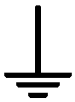
User Safety

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 xiv.

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		

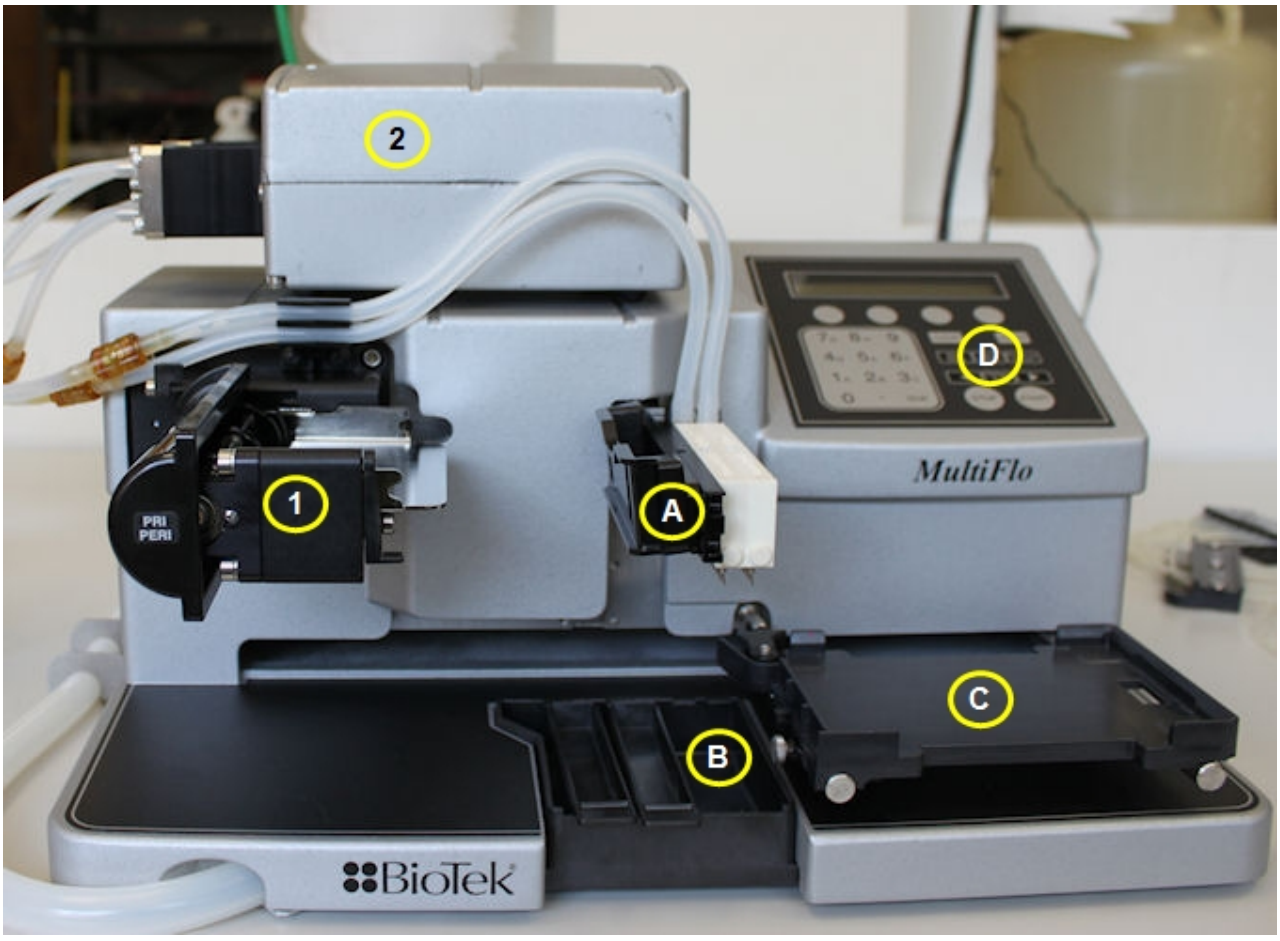
Chapter 1

Introduction

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

Introducing the MultiFlo™ Dispenser

The MultiFlo offers up to three devices in one instrument: one or two peristaltic pump dispensers called the Peri-pump, and a dual Syringe pump dispenser.



	Device/Component	Description
1	Peri-pump Dispenser	Peristaltic, 8-channel dispenser with entirely visible fluid path.
2	Syringe Dispenser	Two distinct syringe-pump dispensers, each with an 8-, 16- or 32-tube manifold.
A	Dispense arm	Holds the Peri-pump's Tip Holder and the Syringe dispensers' manifolds.
B	Priming trough	Waste reservoir for collecting priming fluid for the Peri-pump and Syringe dispensers.

	Device/Component	Description
C	Plate Carrier	Holds standard microplates for processing.
D	Keypad	One of two ways to control the instrument; the other way is LHC.

Features of the MultiFlo

- 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 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.

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 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

MultiFlo Dispenser Comparison

The MultiFlo offers two types of 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.</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>
</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

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

¥ Excluding plate carrier and manifold homing movements.

SB = small bore Syringe manifold

BioStack Compatibility

The MultiFlo 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 MultiFlo, refer to the BioStack Operator's Manual for instructions on configuring the MultiFlo to run with the BioStack. To help you get started: **See Operating with the BioStack on page 66**.

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
Power supply	76077
RS-232 serial cable	75034
USB cable (USB Virtual COM Port Driver Software & instructions)	75108
Priming trough insert (2) for Peri-pump	7182043
Priming trough insert (2) for Syringe dispensers	7182044
Strip plate (12x1)	98265
Screwdriver, Phillips	98268
Stylus: for cleaning Syringe manifold dispense tubes	2872304
10 cc syringe and tubing for Peri-pump cassette maintenance	49919
Shipping brackets (2)	7172073 7212012
Hex wrench: 3/32” for removing syringe pumps	48570
Hex wrench: 1/16” for removing magnets from syringe dispense manifolds	48713
Hex wrench: 7/64" for removing shipping brackets	48169
Peri-pump Reservoir Holder (2) (+2 straps)	7210509
MultiFlo™ Getting Started Guide (and Operator’s Manual on CD - PN 7211000)	7211004

- 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
Special plate carrier for mini-tubes		7212042
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		7210512

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.

Secondary Peri-pump assembly: PN 7210010

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
Non-Autoclavable Syringe Dispenser Module	7210009
Autoclavable Syringe Dispenser Module	7210008
8-Tube (2 x 8 channel) Manifold (1)	7180548S
16-Tube Manifolds (2)	7180543S
32-Tube Small Bore (SB) Manifolds (2)	7180533S

Accessory	PN
32-Tube Large Bore (LB) Manifolds (2)	7180534S
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
Inline Filters (2)	48705
Spare tubing sets (2 - 1/dispenser), autoclavable	7183006
DMSO- & Acetonitrile-safe tubing sets (2 - 1/dispenser)	7183002

Physical Specifications

Labware	
Microplates	96-well standard, half-height, deep; 384-well standard, deep, PCR; 1536-well standard and flanged. The Peri-pump also supports 6-, 12-, 24-, and 48-well plates when special handling instructions are followed. Corning® 96-Well Cluster Tubes (PN: 4410, 4411), called Mini-tubes in this application, are supported using a special plate carrier.
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.
Dimensions (W x D x H)	Approximately 10 3/5 x 16 x 7 ½ inches (27 cm x 41 cm x 19 cm)
Weight (≤)	14 1/4 lb (6.5 kg)
Operating Conditions	10° - 40°C (50° - 104°F)

Hardware & Environmental	
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.

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. Tubes are angled 7 degrees 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

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.

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 MultiFlo™ Microplate 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.

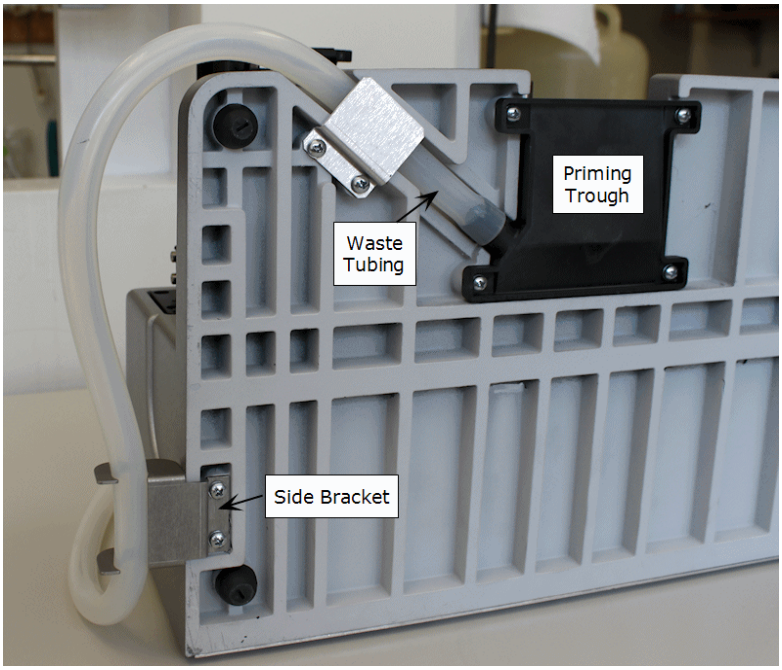
1. Unpack the boxes containing the instrument and other equipment:
   - MultiFlo™ Microplate Dispenser and accessories
   - Dual Syringe Dispenser and accessories
   - Additional Peri-pump Dispenser and accessories
2. Place all packing materials back into the shipping boxes for reuse if necessary.
3. **Syringe Dispenser** models: when the Syringe dispenser is a component of your instrument, review the placement options for it *(as described on page 34)* and decide which one best suits your lab before proceeding with the installation.

Refer to the Package Contents on page 7 to make sure you have all expected equipment.

Install the Waste Tubing

A length of tubing and a bracket to hold it is provided to drain the priming trough into a waste container. It's safer to perform this task before removing the shipping hardware.

Underside of MultiFlo showing priming trough waste tubing



Important note: The waste tubing must lay flat to properly drain the priming trough. The side bracket provided with the instrument supports this goal, but it may not be needed in your lab, depending on where you position the dispenser and its waste receptacle.

You will need:

- Philips head screwdriver (small)
- Waste tubing: 4' provided (PN 7213010)
- Possibly scissors or knife to cut tubing to desired length
- Side bracket provided
- Waste vessel: to capture discarded fluid

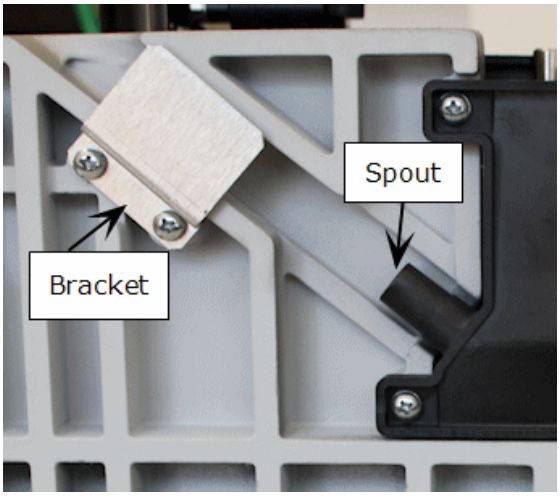
To install the tubing:

Install Side Bracket for Waste Tubing

1. Turn instrument onto its back to access its underside.
2. Remove the two screws in the lower left corner. You will use them to install the bracket.
3. Align the side bracket with the screw holes so its hook opens towards you and use the screws to install the bracket.

You may or may not use the side bracket, depending on where you place the waste vessel.

Attach the Tubing to the Priming Trough



1. Thread the waste tubing under the bracket and onto the priming trough's spout.

Remove the bracket and/or use water or alcohol to help the tube slide onto the spout.

2. Snake the tubing around the instrument to the side bracket.
3. Return the instrument to normal position.

Position Waste Container and Tubing

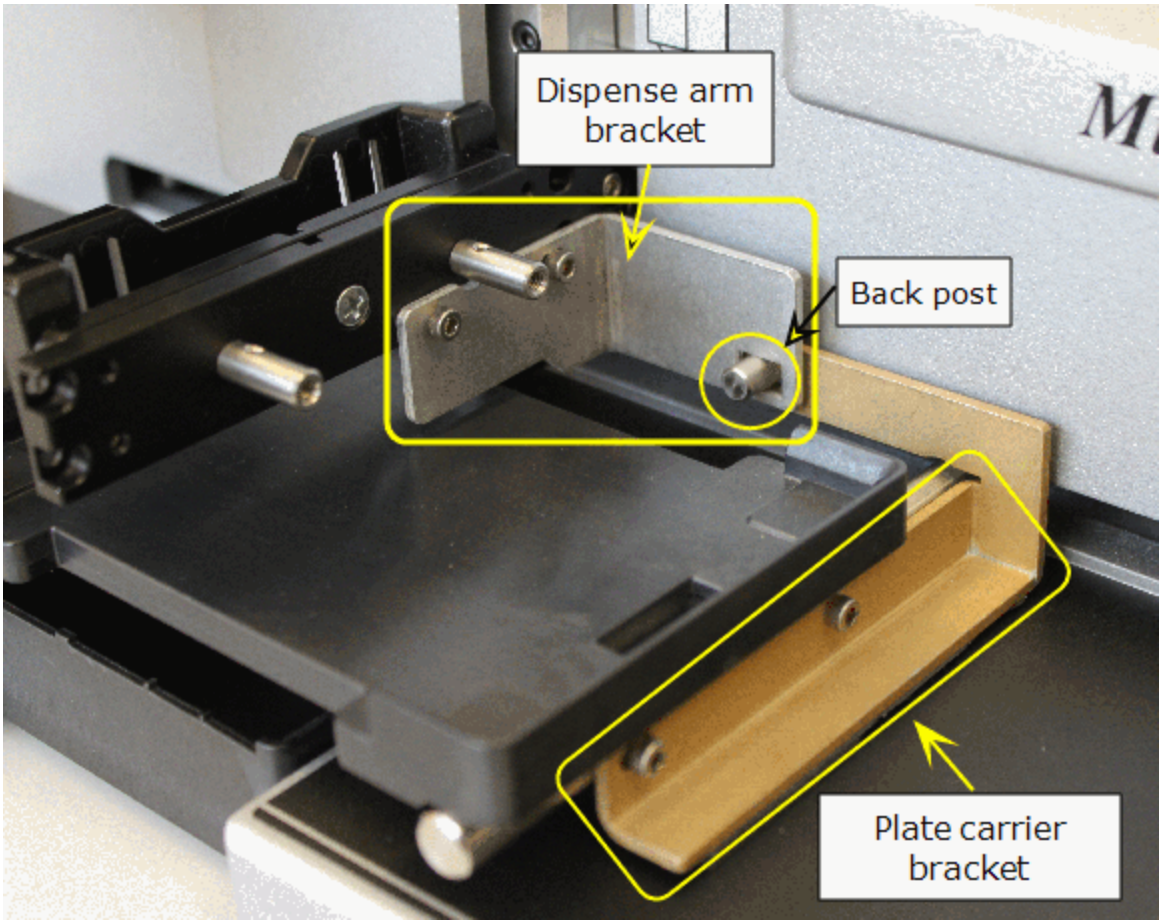
When all the installation steps are completed:

1. Place the waste container under the instrument's work surface.
2. Position the waste tubing to drain into the waste container, cutting it to the optimal length, if necessary.

Next, remove the shipping hardware.

Remove the Shipping Hardware

Two shipping brackets (and a rubber band when a Peri-pump is included) protect the MultiFlo during shipping. After installing the waste tubing for the priming trough, place it upright on a level work surface to remove the shipping hardware. (Store the shipping brackets on the back of the instrument in slots provided for this purpose.)



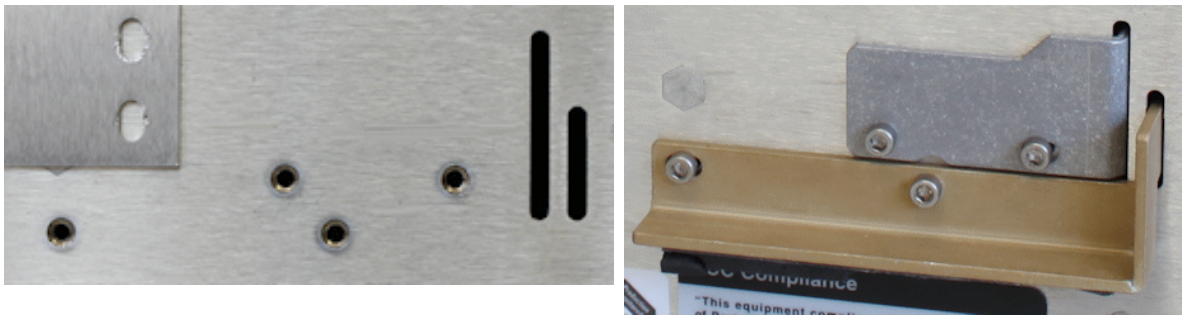
You will need:

- Allen (or hex) wrench taped to plate carrier.

Remove shipping brackets:

1. First remove the dispense arm shipping bracket: use the Allen wrench to remove the two screws that hold it to the dispense arm, lift the arm slightly and slide the bracket forward off the back post.

2. Next, remove the plate carrier shipping bracket: use the Allen wrench to remove the two screws that hold it to the plate carrier. Tilt the bracket downwards slightly, and release it from the post.
3. Attach the shipping brackets to the back of the instrument for storage. They will be needed again if the dispenser must be shipped in the future.



Storage area for shipping hardware on back of instrument

4. Insert the brackets into their respective slots on the back of the instrument, below the fan. Slide the bracket's longest arm into the slot and use the Allen wrench to screw it in place.

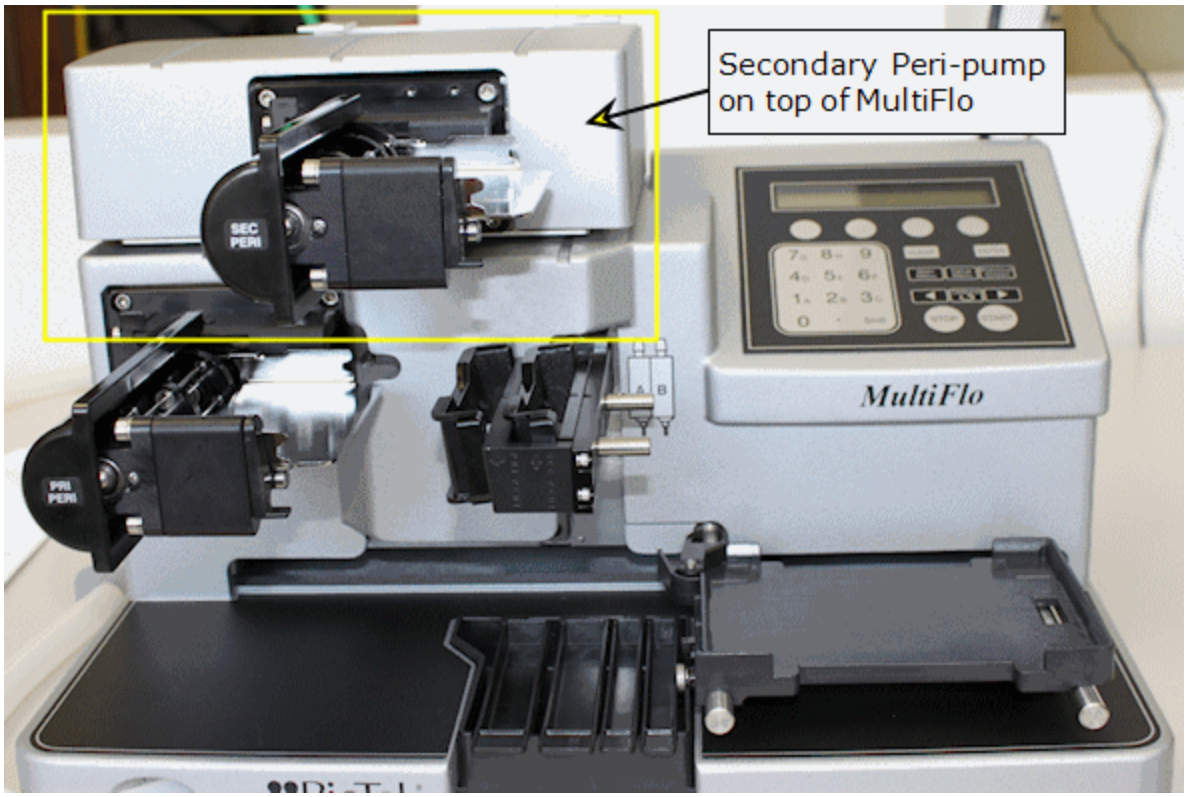
   Store the Allen wrench for future use.

Set Up the Peri-pump Dispenser

Install these items to use the Peri-pump dispenser:

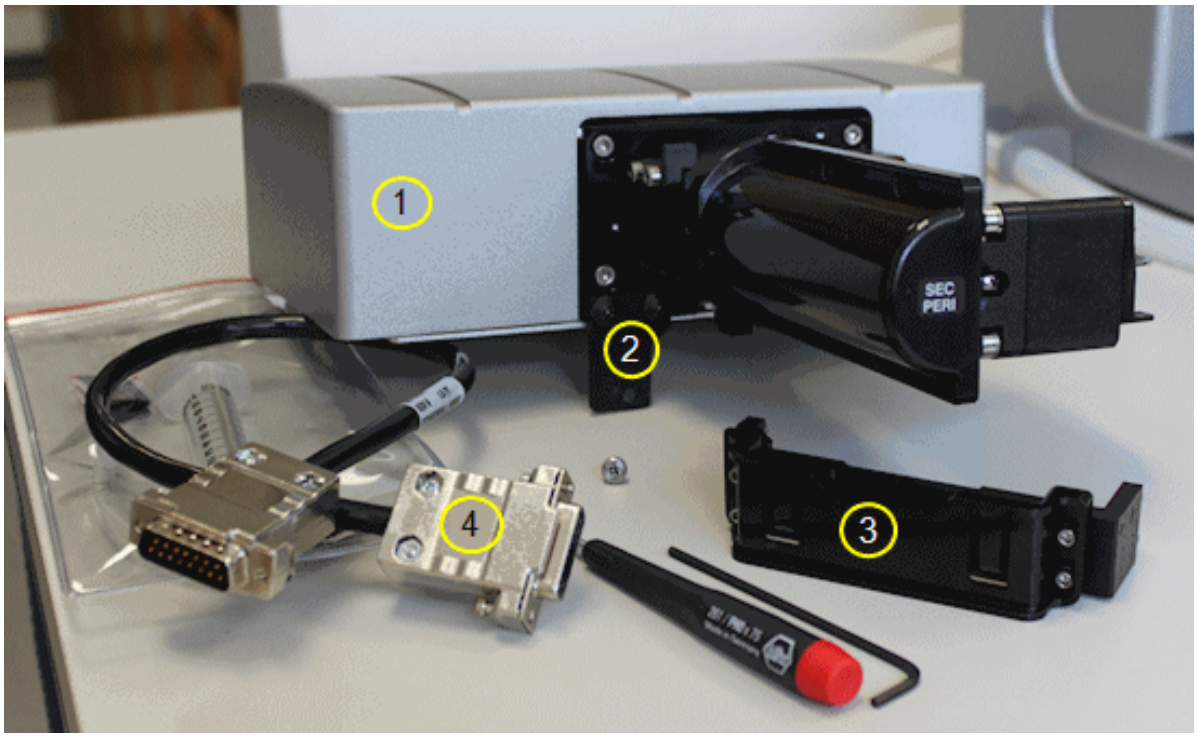
- Install the second Peri-pump, if applicable
- Dispense cassette
- Fluid supply vessel
- (Optional) Prime trough insert

Install the Secondary Peri-Pump



An optional secondary Peri-pump dispenser is available for the MultiFlo. It ships as a kit with its own accessories. Inspect and unpack the shipping container and follow these installation instructions if applicable. Otherwise, skip this part of the installation process.

The secondary Peri-pump dispenser is designed to sit on top of the MultiFlo dispenser. The second pump will be positioned above and slightly right of the primary pump.



Kit components include:

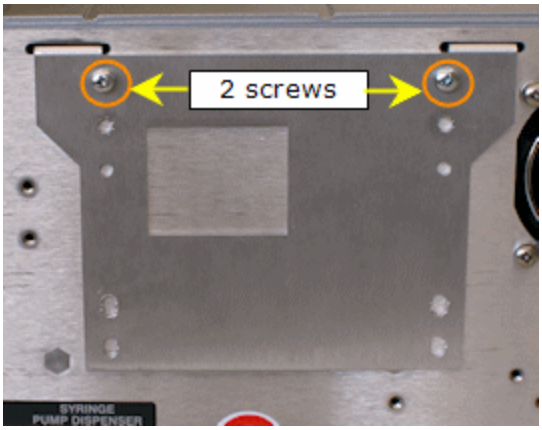
- 1 - additional Peri-pump
- 2 - small tab to secure unit to the instrument
- 3 - additional dispense cassette bracket
- 4 - interface cable to connect unit to the instrument
- Also note the screwdriver, Allen (hex) wrench, and shoulder screw shipped with the accessories.

You will need:

- Second Peri-pump Kit components (shown above)
- Philips head screwdriver (small)
- Small flat screwdriver

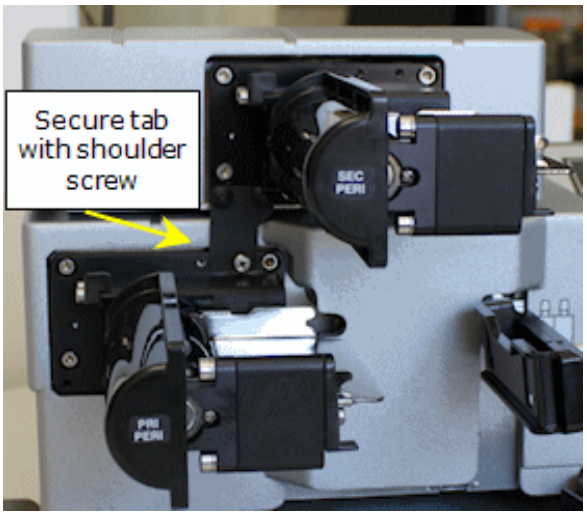
Install the additional Peri-pump:

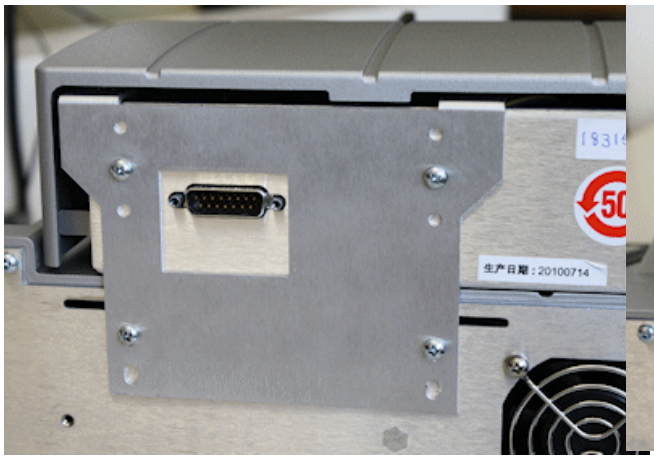
1. First, remove the support bracket stored on the back of the instrument. It will be used to secure the dispenser on top of the instrument. Remove the two screws holding the bracket in place.

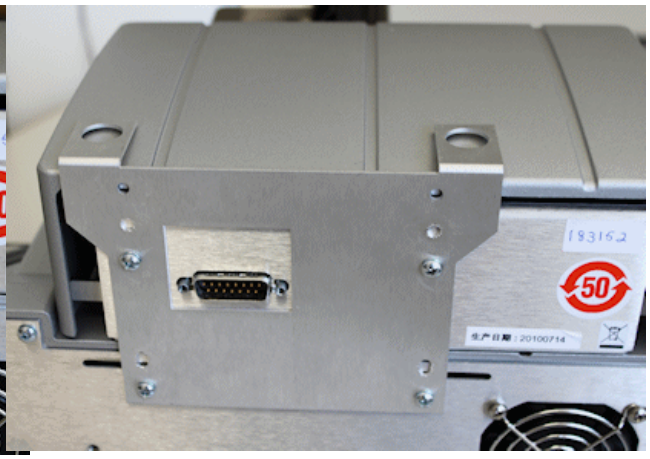


2. Two more screws needed for the installation are shipped on the back of the Peri-pump unit. Remove the two screws and keep with the bracket.

3. Place the second Peri-pump unit on top of the dispenser.
4. Position the dispenser so the screw hole in the small tab attached to its front aligns with the right-hand screw hole on the primary Peri-pump.
5. Use the Philips screwdriver to fully tighten the shoulder screw to ensure the unit is secured.







6. On the back of the instrument, secure the Peri-pump unit in place with the support bracket and the four screws removed from the instrument earlier. Partially tighten the screws until all 4 are in place, then tighten all the screws.

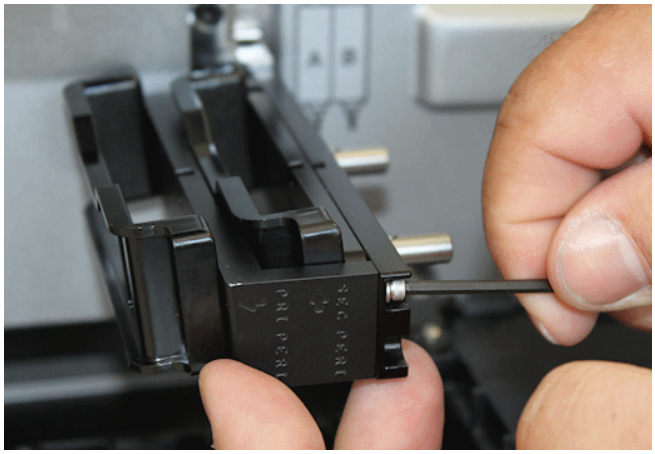
The support bracket can be installed two ways, to provide foot holds for a Syringe dispenser module, or not.
Hide the bracket's foot holds inside the case when a Syringe dispenser will not be installed on top of the second Peri-Pump.

Install the additional dispense cassette bracket:

This task is easier to perform when the cassette bracket is well lit. It's a tight space and the second cassette bracket fits snugly onto the original bracket.

1. First, remove the four screws and washers from the additional cassette bracket using the small Allen wrench supplied in the kit.

2. Slide the plate carrier to the left, out of the way. Lower the dispense arm to a workable position.
3. Use the text on the front of the additional cassette bracket to orient it correctly and place it around the original bracket.



4. Use the four screws to secure the bracket over the existing bracket. Place and partially tighten the screws until all four are in place, then tighten all the screws.

Install cable to connect the dispenser:

1. On the back of the instrument, connect the interface cable to the **Peri-pump Dispenser** port on the back of the instrument and to the port on the back of the Peri-pump unit. Use the small flat screwdriver to secure the cable.

- If you are installing a Syringe dispenser also, you may find it easier to complete that installation before connecting the cable, turning on the instrument, and installing the dispense cassettes.

Turn on the instrument:

1. Plug in the MultiFlo and turn it on (switch on side).
2. A "Hardware Change Detected" message will display. After a few moments the main menu will be shown signaling the installation of the additional Peri-pump:

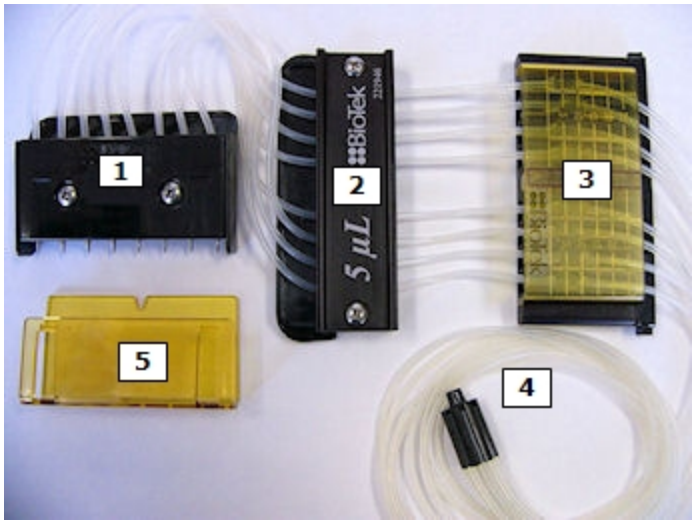
```
PRI-#   SEC-#
RUN   QUICK    DEFINE  UTILS
```

Main Menu

PRI-# represents the primary or original Peri-pump, and SEC-# represents the additional or secondary Peri-pump. The # represents the cassette type. These designations are used in all instrument commands to control the dispenser.

Important: Make sure the installed dispense cassette matches the cassette type number displayed in the menu. Change the cassette type setting on page 29.

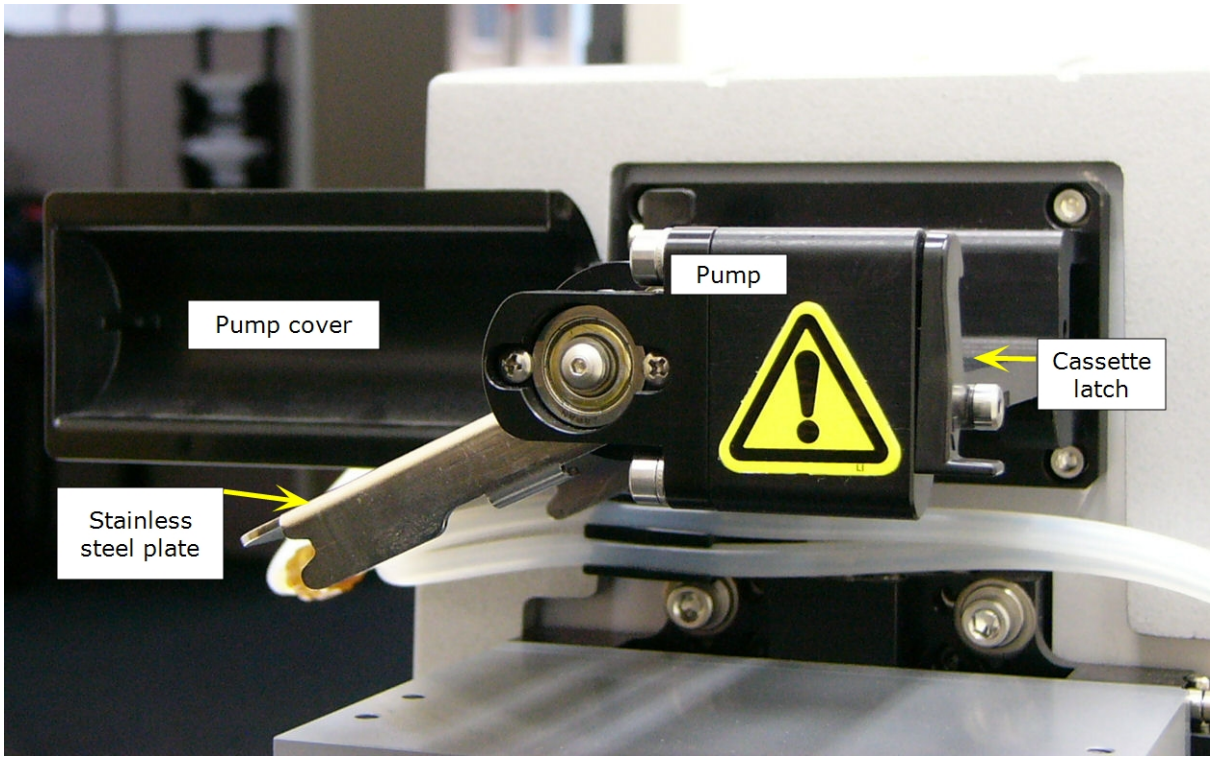
Dispense Cassette Diagram



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



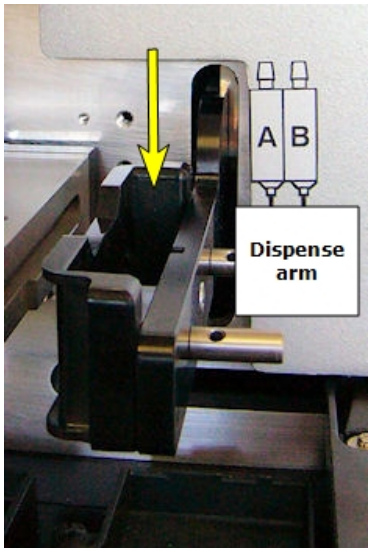
Prerequisites:

- 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.
- When you have two Peri-pumps installed, put the Primary pump's Tip Holder in the left bracket and the Secondary pump's Tip Holder in the right bracket.
- 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.

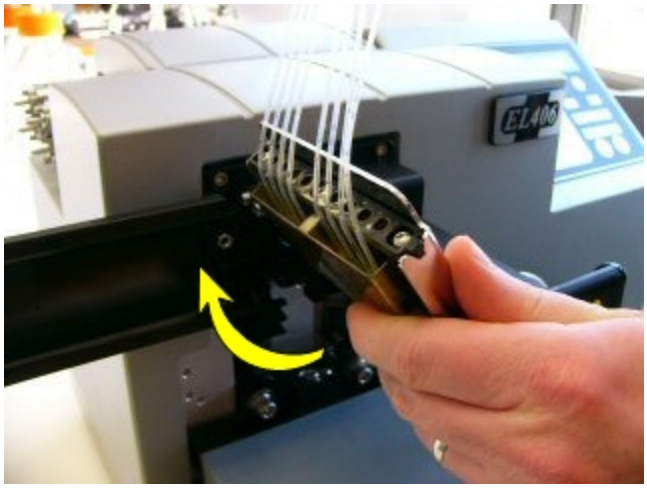
1. Slide the **Tip Holder** into the dispense arm. The Tip Holder's front plate with the BioTek logo or steel plate faces the pump. Make sure the tip holder is level and snapped into place.

- Two Peri-pumps: use left bracket for Primary, right bracket for Secondary.



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**.

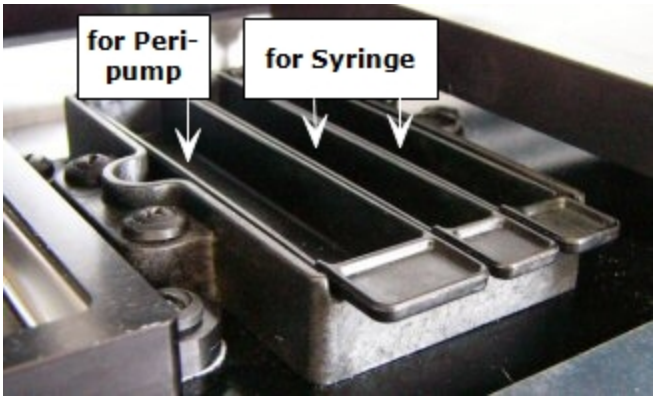
Change the cassette type setting

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

LHC	Keypad
1. Select **Tools>Instrument Utilities** 2. Select the **Peri-pump** tab. 3. Select the correct button for the Cassette Type installed for the primary or secondary Peri-pump, if applicable. 4. Click **Send** to update the instrument.	1. Press **Setup Menu**. 2. Select **PERI**. 3. Select **PUMP** and Primary or Secondary, if applicable. 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 101)* to automatically update the instrument's Cassette Type setting when you run a protocol. For advanced users with well organized procedures, the MultiFlo 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



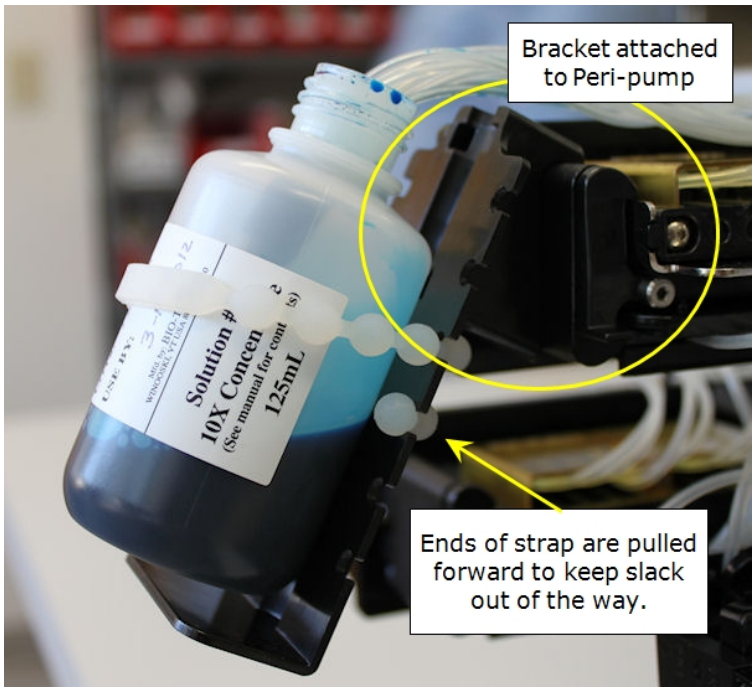
The MultiFlo ships with special reservoirs that fit into the dispensers' priming trough to capture expensive reagent after priming, rather than discarding it.

Up to three Prime Trough Inserts are provided:

- PN 7182043 (2) for the Peri-pump dispenser holds approximately 12 mL
- PN 7182044 (2) for the Syringe dispensers holds approximately 6.5 mL
- PN 7182109 large priming trough insert, holds approximately 40 mL

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

Peri-pump Reservoir Holder



Peri-pump Reservoir Holder attached to Peri-pump

MultiFlo models with a Peri-pump dispenser include the convenient Peri-pump reservoir holder (PN 7210509). The simple bracket clips onto the front of the Peri-pump to hold a variety of vessels.

You can significantly reduce the dead volume, saving precious fluid, by cutting the cassette tubing to make a shorter fluid path. **See Shorten the Dispense Cassette Tubing below**.

The reservoir holder has two components, a bracket and a strap.

1. Clip the bracket onto the front of the Peri-pump. When properly installed the bracket hides the hazard label.
2. Place the supply vessel (reservoir) on the bracket and wrap the strap around both the vessel and the bracket: fit the strap's molded balls into the round slots.

Depending on the size of the vessel, it may be necessary to tuck the ends of the strap into the bracket, too, to keep the slack out of the way.

- Important: Always remove the tubing from the fluid before releasing the tension on the cassette or changing cassettes.

Shorten the Dispense Cassette Tubing

Shortening the fluid path reduces the dead volume when dispensing, which helps preserve expensive reagents. BioTek offers the Peri-pump Reservoir Holder above as

an accessory to the Peri-pump dispenser for this purpose. The cassette tubing can be shortened by as much as 10½ inches (26.67 cm) when using the reservoir holder.

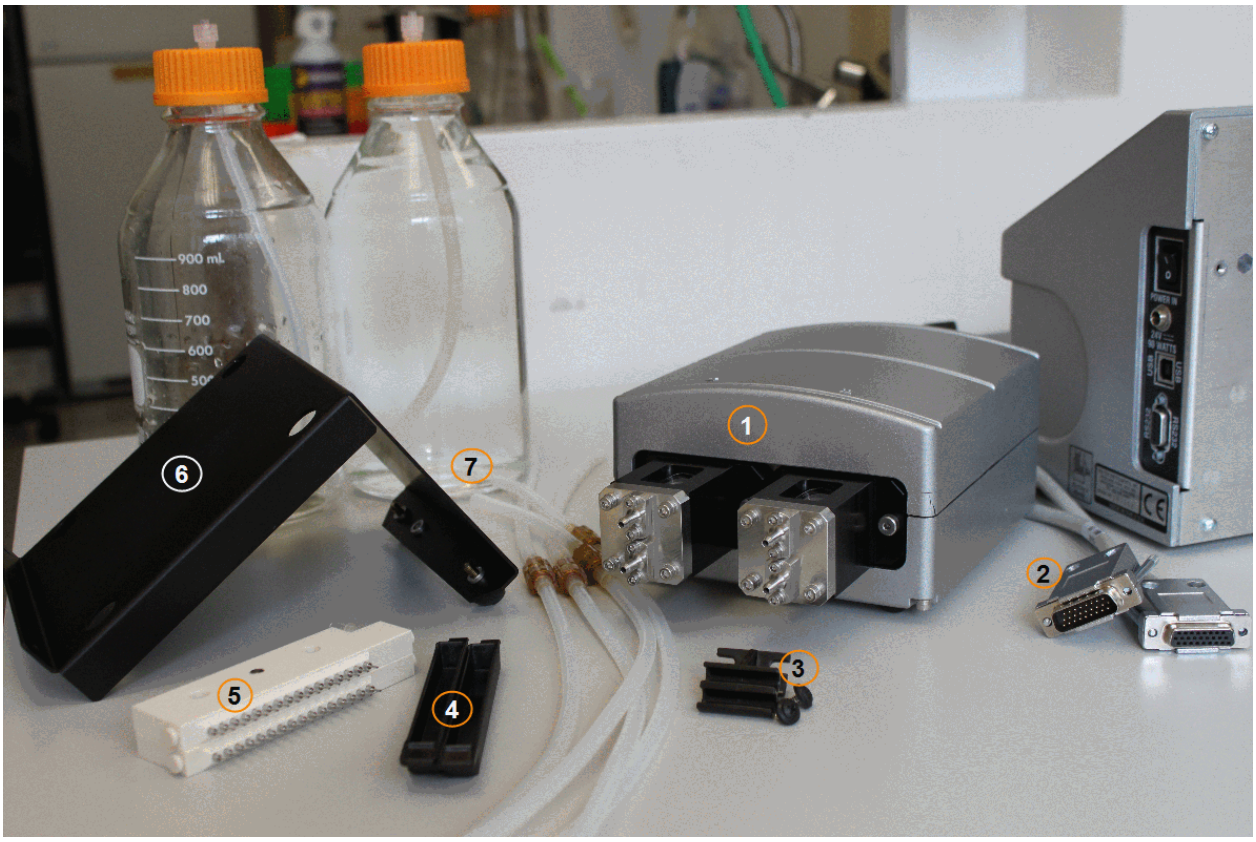
Recommendations for cutting the tubing:

- Prepare a clean, lint-free work area to lay out the cassette;
- Remove the **Tube Organizer**;
- Use a ruler as a guide to determine where to cut the tubing;
- Cut one tube at a time using a razor blade, Exacto® knife, or sharp scissors; make as clean a cut as possible;
- Cut the tubing as evenly as possible, limiting the difference between the tubes to less than 1/4" (6.35 mm).

To may make this job easier, use the cassette's packaging to hold the cassette parts, keeping them out of your way.

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.



- 1 - Syringe Dispenser module (autoclavable)
- 2 - interface cable
- 3 - tubing bracket
- 4 - priming trough inserts
- 5 - dispense manifolds
- 6 - shelf
- 7 - supply bottles and tubing with check valves
- Not shown: thumbscrews (2), large priming trough insert.

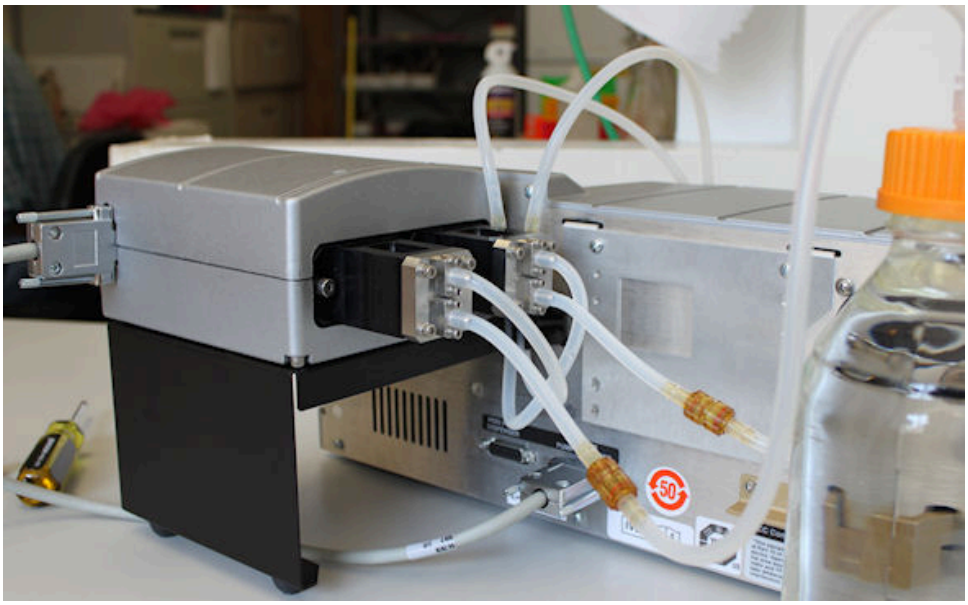
Install the Syringe Pumps

Review the placement options for the Syringe dispenser and follow the applicable instructions:

Syringe Dispenser Placement Options on next page

Syringe Dispenser Placement Options

There are two major placement options for the dual Syringe dispenser module: in back of the instrument on a shelf provided for the purpose or on top of the instrument. When installing the Syringe dispenser on top, placement of the tubing bracket varies slightly depending on how many other dispensers are installed.



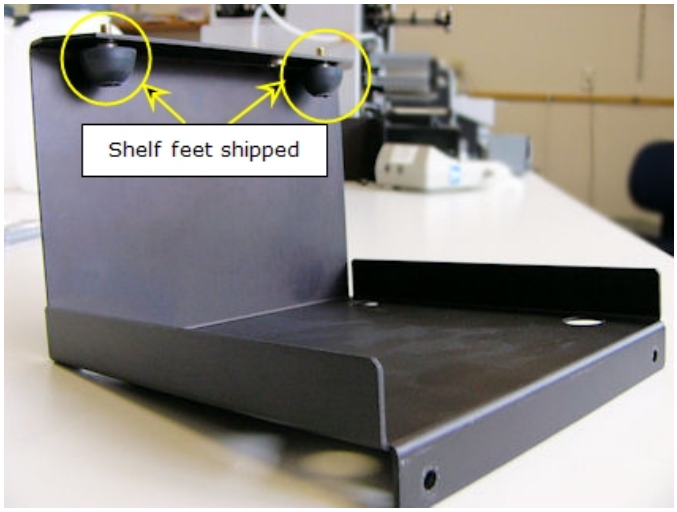
Install the Syringe Dispenser on Back Shelf on the facing page

Follow the installation instructions for your preferred placement of the dispenser:

Install the Syringe Dispenser on Top on page 37

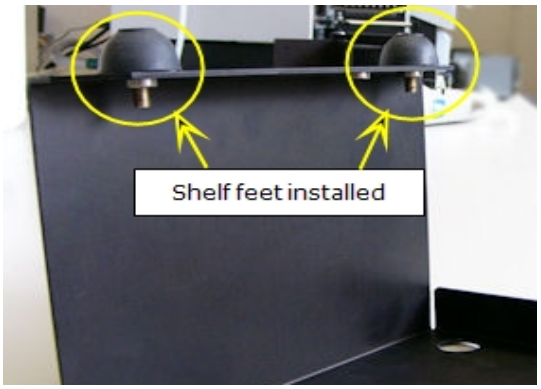
Install the Syringe Dispenser on Back Shelf

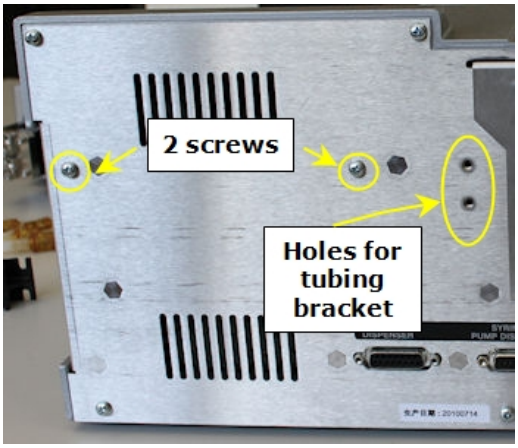
Follow these instructions to install the dual Syringe Dispenser module on a shelf attached to the back of the MultiFlo.



First, reposition the shelf's feet:

1. Remove the shelf shipped with the Syringe dispenser from its plastic bag (PN 7212038).
2. Unscrew the rubber feet that are shipped in the wrong position.
3. Install the feet in the correct position: screw the feet into the two outer holes on the bottom of the shelf. (The third hole is for BioStack integration.)
4. Remove the two screws on the left side of the back of the instrument.
5. Use the screws to install the shelf.





6. Install the tubing bracket (using the black-capped screws) in the two holes next to the shelf. First insert the bottom screw part way. Position the bracket over the screw and tighten both screws.

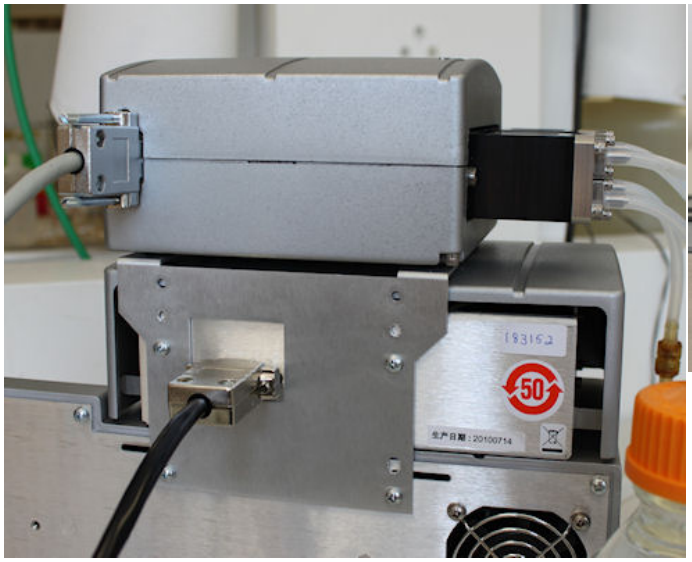
7. Put the syringe pump module on the shelf.
8. Plug the 26-pin high-density cable into the back panel of the MultiFlo in the port labeled **Syringe Pump Dispenser**. Plug the other end into the syringe pump unit.

Next steps:

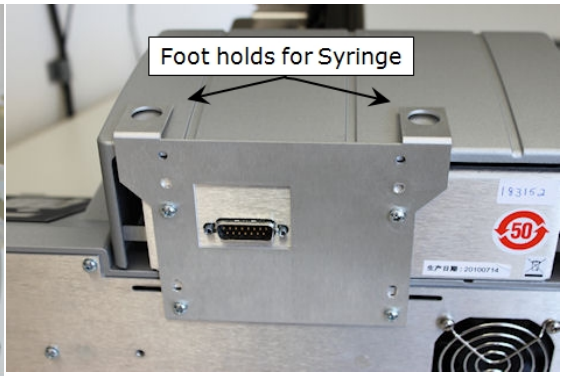
- Update the instrument to use the Syringe Dispenser on page 44
- Install Tubing and Manifolds for Syringe Dispenser on page 39

Install the Syringe Dispenser on Top

Follow these instructions to install the dual Syringe dispenser module on top of the instrument.



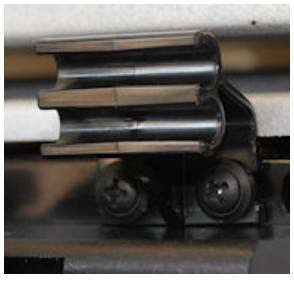
Syringe dispenser on top of secondary Peri-pump



Support bracket with foot holds for the Syringe dispenser.

The MultiFlo ships with a support bracket attached to the back of the instrument. The support bracket has foot holds for the Syringe dispenser. The bracket's flexible design supports either one or two devices, the secondary Peri-pump and the Syringe dispenser, alone or in combination.

1. Remove the support bracket from the back of the instrument by releasing the two screws that hold it in place.
2. Align the bracket with the screw holes on the back of the instrument so that the foot holds sit above the instrument or second Peri-pump's top.
3. Install the tubing bracket in the appropriate location using the two black-capped screws:

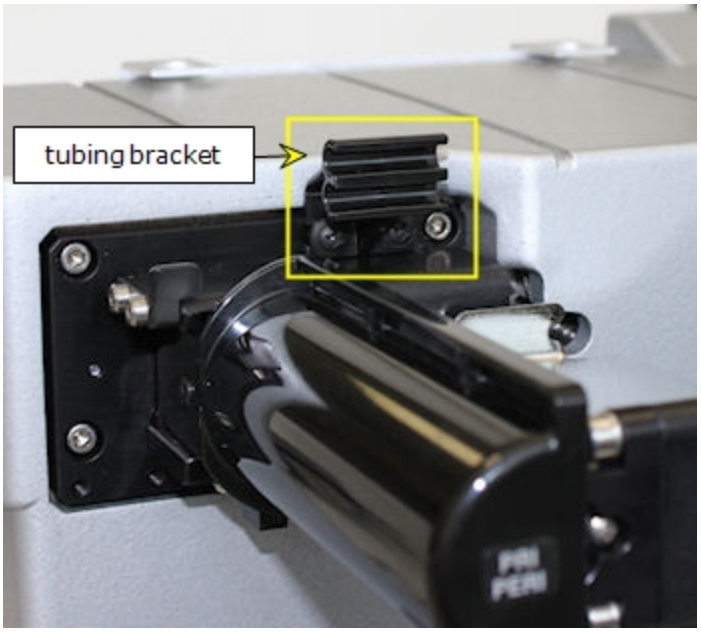


Tubing Bracket

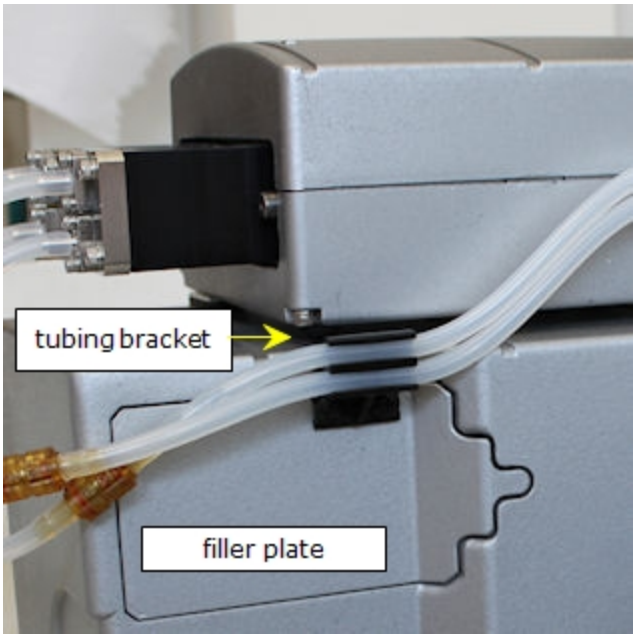
When installing the Syringe dispenser on top of the instrument, the placement of the tubing bracket varies slightly depending on how many other dispensers are installed. Always position the bracket as close as possible to the syringe pump unit.

1 Peri-pump:
Install the bracket using the two empty screw holes on top of the Peri-pump.

2 Peri-pumps:
Install the bracket using the screw holes on top of the **secondary** Peri-pump, the pump closest to the Syringe.



Syringe Only
(no Peri-pump)
Remove the two screws in the filler plate on the front of the instrument and replace them with the tubing bracket.



4. Plug the 26-pin high-density cable into the back panel of the MultiFlo in the port labeled **Syringe Pump Dispenser**. Plug the other end into the syringe pump unit.

Next steps:

- Update the instrument to use the Syringe Dispenser on page 44
- Install Tubing and Manifolds for Syringe Dispenser on the facing page

Install Tubing and Manifolds for Syringe Dispenser

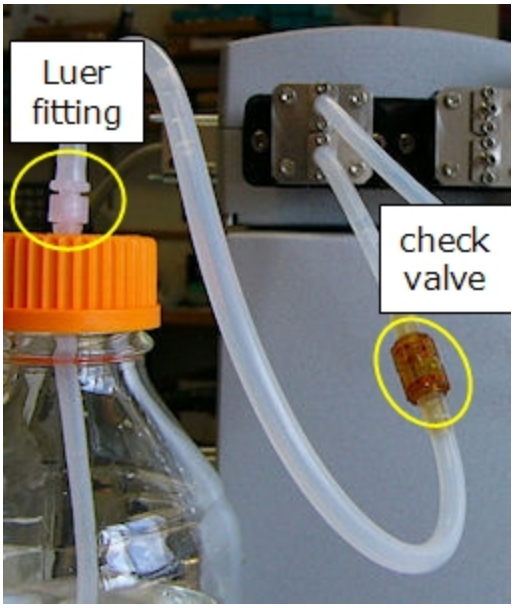
For each dispense pump, a set of two tubes with check valves and two supply bottles are provided. The 32-tube dispense manifolds also ship with an optional inline filter.

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

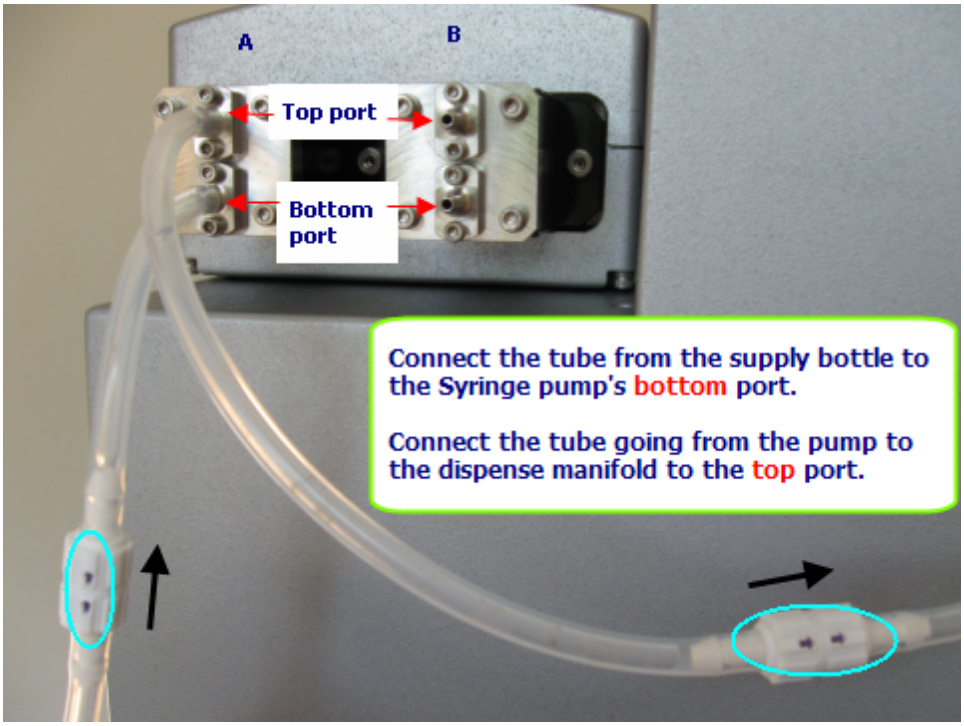
- Rinse all bottles with deionized or distilled water before use to eliminate particles that may have entered during packing or unpacking.
- Place the supply bottles on the same horizontal plane as the instrument. This ensures optimum pump performance.
- Make sure the tubing is not crimped during installation.

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 42)*. 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.

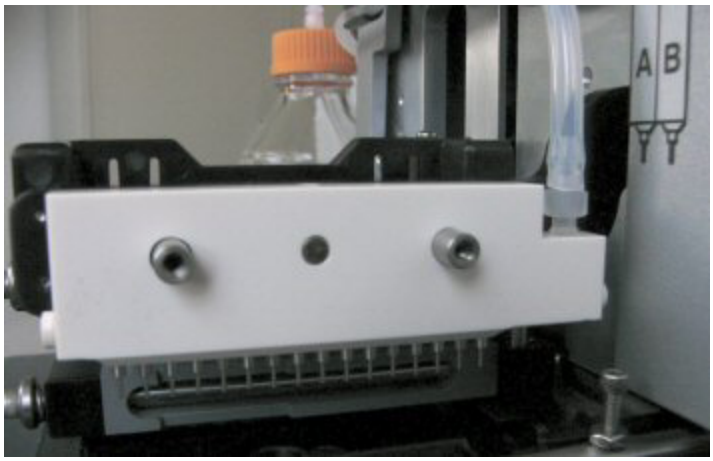


- Make sure the flow-direction arrows point in the direction that the fluid moves.



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.

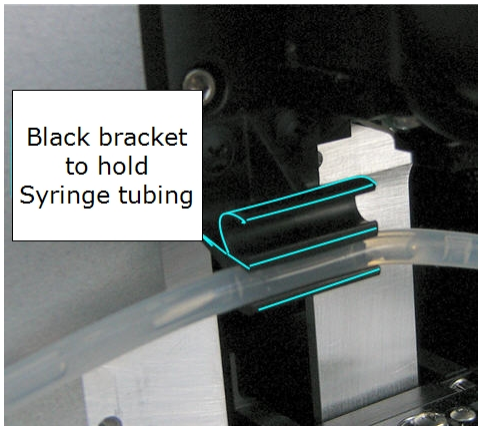
4. Slide the manifold onto the two posts on the dispense arm with the tubing end closest to the instrument. Except for the dual 8-channel manifold that has two manifolds in one block, install Syringe A's manifold first. Syringe B's manifold must be installed after Syringe A's.

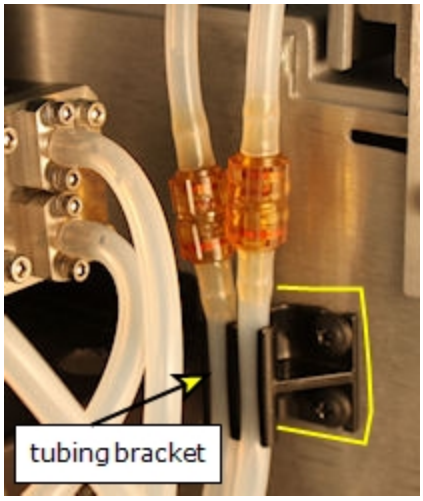


5. Connect the supply tube to the dispense manifold.

- 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 108)*.





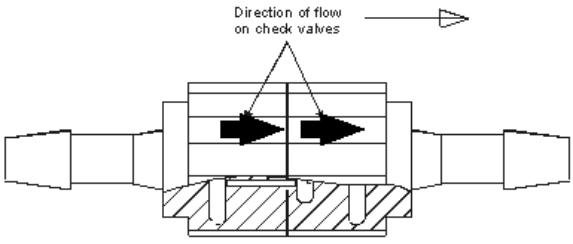
6. Make sure the manifold has enough slack in the tubing to move down to the priming trough and then press the tubing into the black tubing bracket to keep the tubing out-of-the-way.

> ▪ When the Syringe is installed on the back shelf, you must temporarily move the pump unit to get access to the tubing bracket.

7. Repeat the tubing and manifold installation for Syringe B.
8. (Optional) Insert the thumbscrews into the posts that hold the manifolds. (Magnets keep the manifolds attached to the dispense arm. The thumbscrews are provided in case the magnets interfere with operation, e.g. for magnetic bead assays.)

> ▪ Important! You must Update the instrument to use the Syringe Dispenser on page 44to tell the instrument which manifold is installed.

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 153** for instructions.

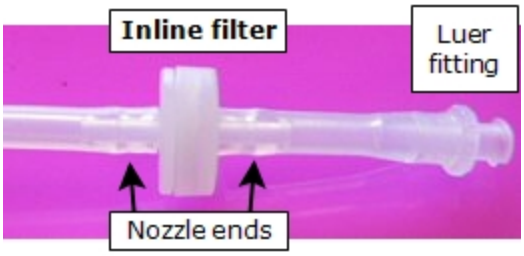
Make sure the flow-direction arrows on the check valves point toward the pump from the supply vessel, and towards the dispense manifold from the pump.

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 Software/Connect to Computer

If you purchased BioTek's Liquid Handling Control™ (LHC) Software to control the MultiFlo 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 MultiFlo:

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

1. Connect the power cable to the power supply.
2. Plug the power supply cable into the power socket in the side panel of the MultiFlo.
3. Insert the three-prong plug into an appropriate receptacle.

Define Instrument Settings

LHC Users Only

 When using the LHC to control the MultiFlo, 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 MultiFlo.
2. Specify the COM **Port** used to connect the MultiFlo 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**.

Update the Instrument to use the Syringe Dispenser

An important part of installing the dual Syringe Dispenser is updating the instrument's basecode (internal software) with its calibration data. A data sheet is affixed to the bottom of the Syringe pump unit and to the front page of the installation instructions. Enter the calibration values that match the installed 8- or 16-tube manifold; for 32-tube manifolds enter either data set.

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 MultiFlo (define the correct COM port). 3. Select **Tools>Instrument Utilities>Syringe Dispenser**.	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

LHC	Keypad
4. Under Syringe Dispenser Assembly, specify 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 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.	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. 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 MultiFlo 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 MultiFlo for the first time, verify that it is operating properly.

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

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 MultiFlo.
2. Define the COM **Port** used to connect the MultiFlo 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 186 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 7210512) for the MultiFlo 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 57. The guidelines include necessary steps to perform before running a protocol, and issues to consider when creating or editing protocols.

Repacking the MultiFlo

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.

- Decontaminate the instrument before returning it: **See Decontamination on page 146**.

If the original packing materials have been damaged or lost, contact BioTek to order replacements. Part Numbers: 7213006 for the main instrument, and 7213008 for the secondary Peri-pump and 7213007 for other accessories, if applicable.

Prepare the instrument for shipping bracket installation:

1. Remove the Peri-pump's cassette and the Syringe dispensers' manifolds, if applicable.
2. Uninstall and repack separately the Syringe pump and its accessories, if applicable.
3. 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:

- Repacking illustration 1: install the shipping brackets.
- Repacking illustration 2: put the instrument into shipping boxes.
- Repacking the Syringe Dispenser (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

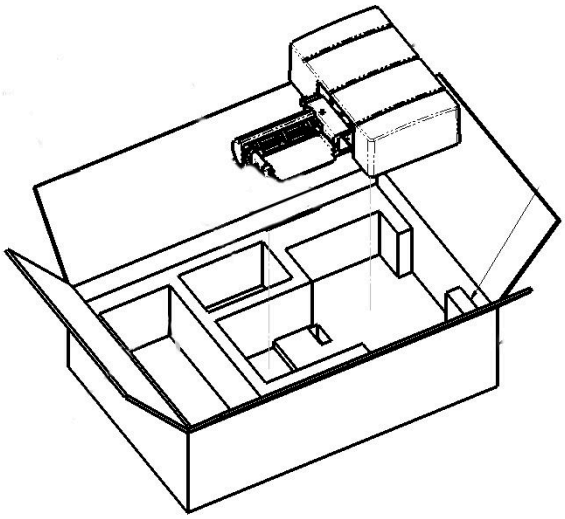
MultiFlo Repacking Illustration 1

> ▪ 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.

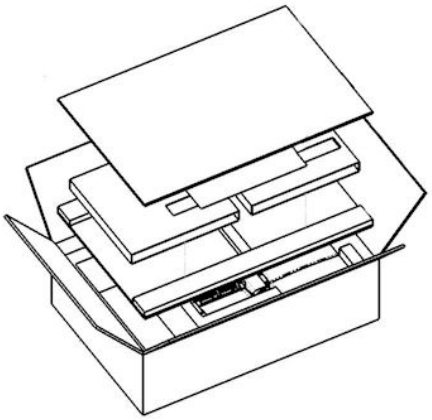
Multiple accessory modules are available for the MultiFlo. Foam blocks are used as space fillers in the packing containers when the modules, like the secondary Peri-pump, are not shipped.

Uninstall the Secondary Peri-pump

1. Reverse the installation procedure: Install the Secondary Peri-Pump on page 21 to remove the Secondary Peri-pump from the top of the MultiFlo.
2. Wrap a rubber band around the pump itself.
3. Place the module in the plastic bag and put it in its shipping box.

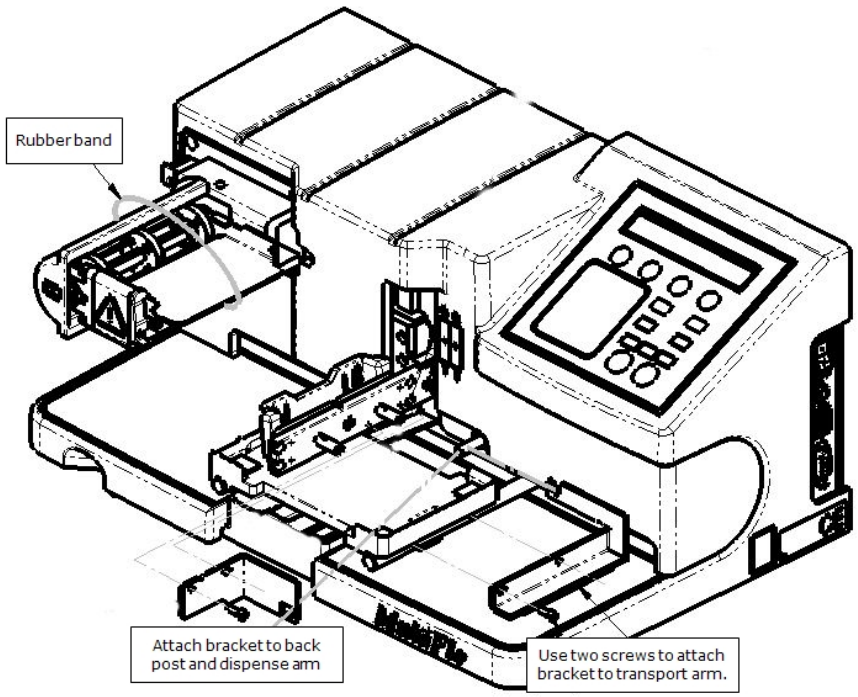


4. Pack other accessories and the power cord in the box.
5. Repack the cardboard and foam shipping materials to secure the items in place.
6. Tape the box closed. This box will fit into the box with the instrument.

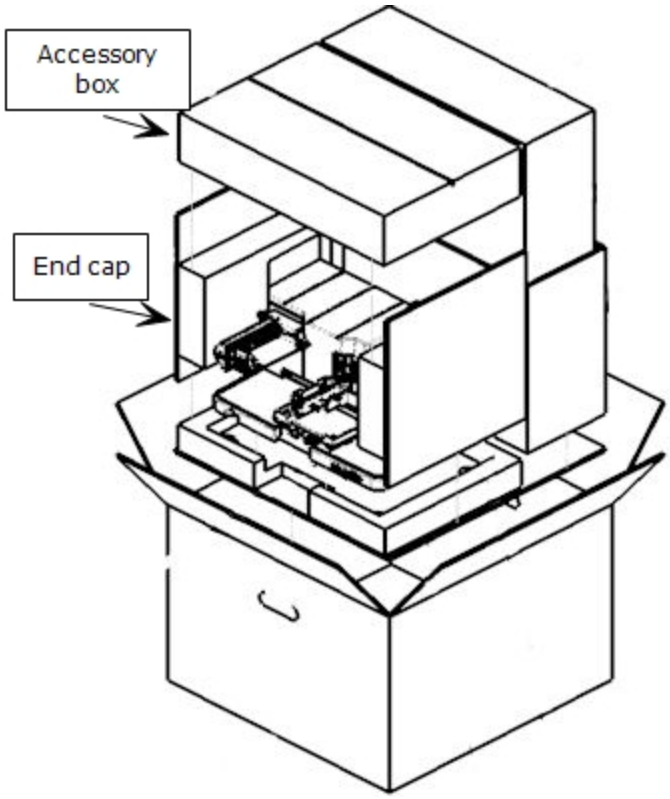


MultiFlo Repacking Illustration 2

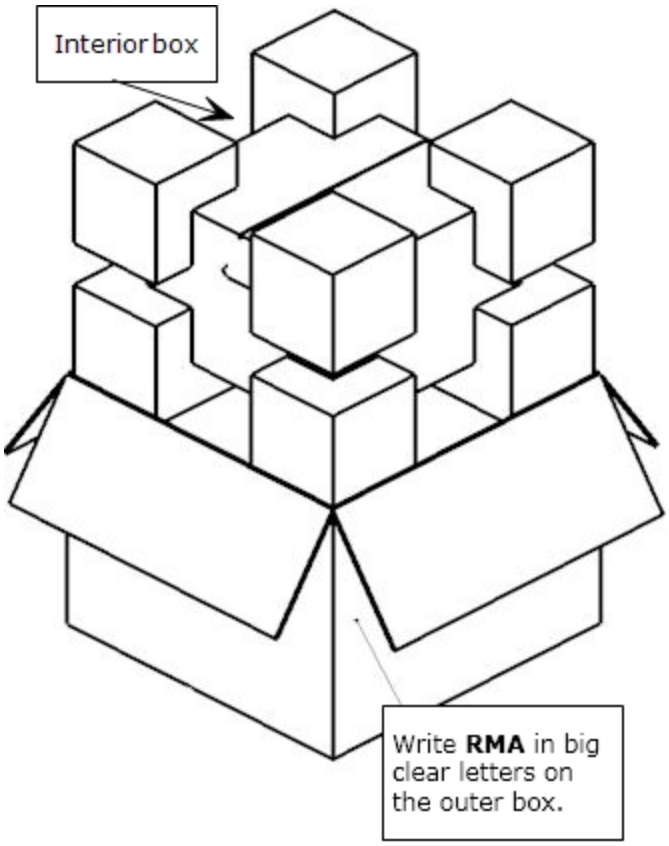
1. Reverse the steps described to Remove the Shipping Hardware page 19. First remove the two shipping brackets from the back of the instrument, then install them.
2. Put a rubber band around the Peri-pump.



3. With the shipping hardware installed, sit the instrument in the foam edged box bottom.
4. Place the end caps on either side and put all items inside the interior shipping box.
5. Place accessory boxes inside interior box.
6. Tape the box closed.

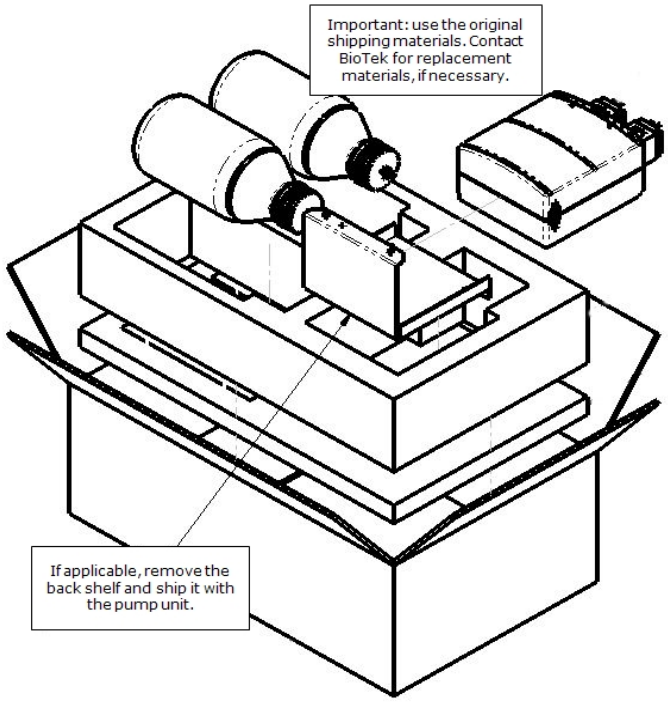


7. Put the eight foam corner blocks on the interior box and put it inside the outer box.
8. Tape the outer box closed.
9. Write "**RMA**" in large, clear letters on the outer box.
10. Include the RMA number in the address label.

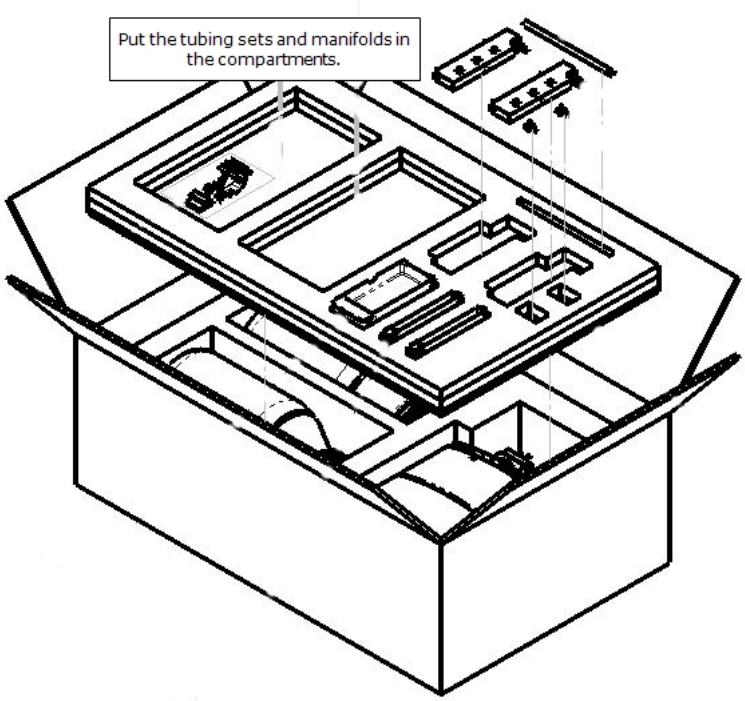


Repacking the Syringe Dispenser

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.



Chapter 3

Operation

This chapter provides instructions for controlling the MultiFlo.

Basic Operation

Two ways to control the MultiFlo

You can control the MultiFlo 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 80.
- Keypad instructions begin on page 68.

Two ways to dispense fluid to a plate

The MultiFlo 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 70**.
- **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 58**.

Turn On the Instrument

To turn the instrument on or off, press the On/Off switch on the side panel:

| = **On** ○ = **Off**

The MultiFlo performs a self-test.

- If the test passes, the Main Menu will appear and the instrument is ready for use.
- If the self-test fails, the MultiFlo will beep and display an error code. Make sure nothing is obstructing the movement of the plate carrier and the devices and restart the instrument. If the error persists, look up the code in **Appendix A: Error Codes** of the operator's manual, shipped on CD.

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 124. Enable **AutoPrime** to keep tubes from clogging.

Prime the tubing to remove air bubbles

- **See Recommended priming volumes for the Peri-pump on page 98**
- **See Recommended prime volumes for the Syringe dispensers on page 104**

Peri-pump

- **See Release the tension on the dispense cassette on page 99**

Best Practices for all MultiFlo Devices

- Fill the supply bottles with sufficient fluid.
- 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.
- 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.

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 60**.

To run a defined protocol:

LHC	Keypad
1.  Select **Open** and locate the MultiFlo folder. 2. Open the MultiFlo folder to access the more folders. Important: Be sure to Customize the Predefined Protocols on the facing page	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: 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 71.

Liquid Handling Control™ (LHC) Software

Launch the LHC software to create or modify protocols, see page 80.

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 MultiFlo 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 **MultiFlo** 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 83.
7. Save the protocol.

Predefined Protocols Listing

Maintenance Protocols

Daily Maintenance	Description
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. (P2 protocols are for the secondary Peri-pump)

Periodic Maintenance	
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.
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.

QC (Quality Control) Protocols

Manifold-Specific	
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. (P2 protocols are for the secondary Peri-pump)

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 59)* 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.

Protocol Parameters Tables

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>
<tr><td></td><td>Plate type:</td><td>Select the plate type, and optionally, limit the columns dispensed to. For high density plates,</td><td>96</td></tr>
</table>

Step	Option	Description/Values range	Default values
		skipping certain "Rows" sections is supported, see Peri-pump Dispense Pattern on page 94.	
	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
	Pump delay:	0-5000 msec.	0

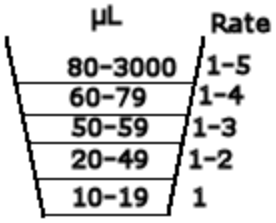
Step	Option	Description/Values range	Default values
		When dispensing highly viscous fluids, the tubing's check valves perform more slowly. Delaying the syringe pump sufficiently to allow the specified amount of fluid to pass through the check valves before being pumped into the syringe has been shown 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
	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 and number of pre-dispenses.	10 µL 2 cycles

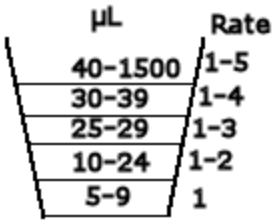
Syringe Dispenser Flow Rates:

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 87 for default Z-axis values or dispense heights.

Operating with the BioStack

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

LHC Control:

- LHC users: connect both the BioStack and the MultiFlo to the computer and control them with the LHC.
- The LHC lets you design protocols that integrate BioStack controls with MultiFlo 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 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 Dispense **Prime** options. This is recommended especially prior to processing plates, to remove air from the tubing.

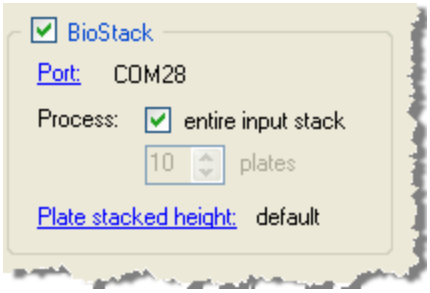
> ▪ Only one of the MultiFlo's communication ports can be used at a time: you can plug in either the USB cable to connect it to the PC or the serial cable to connect it to the BioStack (but not to both at the same time).

Install and Align the BioStack:

1. Set up the BioStack according to instructions in your BioStack Operator's Manual to interact with the MultiFlo. Connect it to the:
   - Host computer (PC) when using the LHC to control the MultiFlo.
   - MultiFlo when using the keypad to control the instrument.
2. Align the BioStack's gripper with the MultiFlo'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 MultiFlo 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 MultiFlo. BIOSTACK2WR: Lift the BioStack's gripper before turning it on.

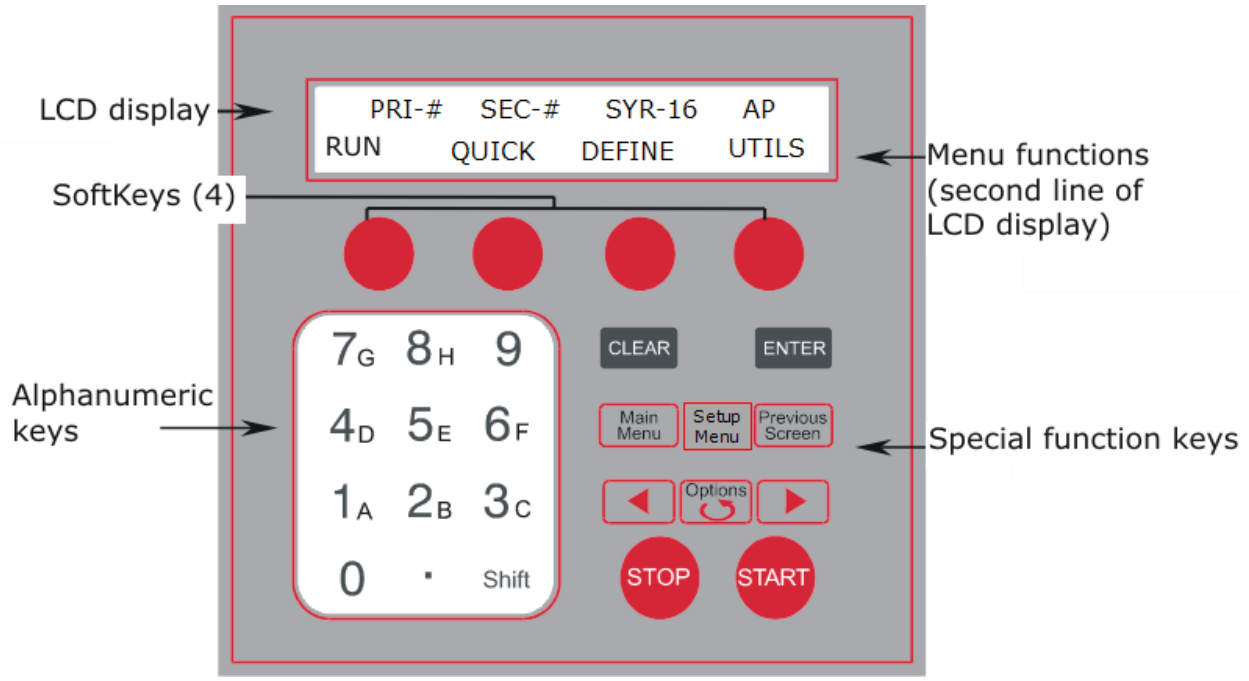
Robotics integrators: CAD drawings of the physical dimensions of the MultiFlo are available upon request. Contact BioTek customer service.

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

Introducing the MultiFlo Keypad

The keypad on the MultiFlo™ Microplate 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 60**.
- **QUICK** – leads to the Quick Dispense menus. Depending on the devices installed, press **Pump** to scroll to the PRI or SEC for Peri-pump, and when the Syringe dispenser is installed, SYR-A, SYR-B, or SYR-BOTH quick dispense menus, if applicable:
- **See Quick Dispense (Keypad only) on page 70**:
  - **Peri-pump Dispenser** (PRI or SEC) options:
    - First, make sure the PRI-# and SEC-# matches its installed cassette type. If not, change the setting: **See Change the cassette type setting on page 29**.

- 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**.

◦ **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 73** and the Plate Types Table on page 87 to learn about the types of microplates supported.

- **DEFINE** – leads to the protocol creation and editing mode: Create or Edit a Protocol on page 71.
- **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.

"**Hardware Change Detected**" - you may need to update the instrument's settings when this message is shown. See Define Instrument Settings on page 44.

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 PRI or SEC for Peri-pump (for primary and secondary pumps), 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 MultiFlo 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.
- **PRI-# or SEC-#** is the Peri-pump cassette setting. Make sure it matches the currently installed cassette: **See Change the cassette type setting on page 29**. Refer to instructions for physically changing the cassette: Install the Dispense Cassette on page 27.

  > ▪ 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.
- **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.

- **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 73**.

> ▪ **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:

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 62 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 MultiFlo, depending on the currently installed hardware, can process 96-well, 384-well, and 1536-well plate types. Dispensing to a part of the plate is limited by the foot print of the hardware. 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 Dispense

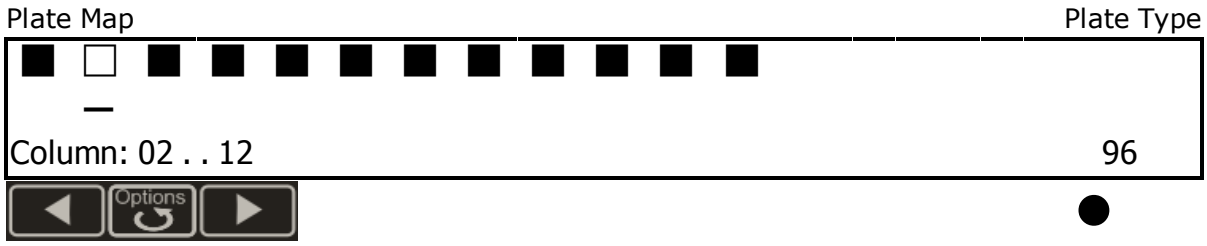
- When defining a quick routine, select **Plate** with the Soft-key to set the plate type, and to define the plate map.

Protocols

- 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):
  - **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.

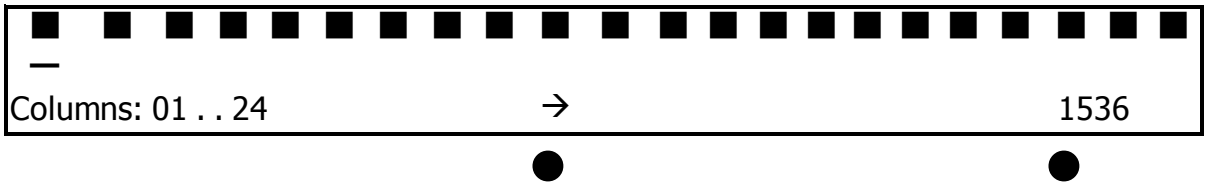


The two-line display changes to show a representation of the current plate type's columns in the top line. The display shows each column as a filled or empty square; empty columns will not be dispensed to. For the current plate type, shown in the right corner, the left corner shows the selection range,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

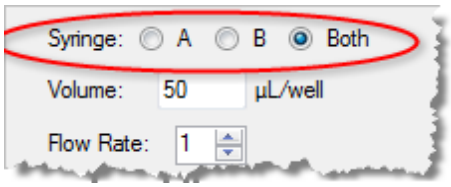


1536-well plates require two screens to show all 48 columns. Select the → key to toggle between the two screens to select the columns to dispense to.

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.

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 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 93** .

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.

To specify a shake period:

- **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 71

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.

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.

How to copy a protocol

You can save significant time creating protocols by copying a protocol that shares some of the same protocol parameters and then editing the copy to meet your needs.

LHC users: Open the protocol you want to copy and select **File>Save As**. Assign unique file and protocol names to it. Consult the Help to learn more.

Keypad users:

1. Select **DEFINE** at the main menu.
2. Select **Copy**.
3. Choose the type of protocol you want to copy.
4. Use the **Options** key or enter the protocol number to select it.
5. Enter a unique name for the new protocol you are creating.
6. Select **Yes** to copy the protocol. Then, you can edit the protocol, as needed.

How to delete a protocol

You can delete protocols to prevent other users from running them.

LHC users: use Windows Explorer or My Computer to delete the protocol file from your PC.

Keypad users:

1. Select **DEFINE** at the main menu.
2. Select **DELETE**.
3. Use the **Options** key or enter the protocol number to select it.
4. Select **Yes** to delete the protocol.

Define Instrument Settings (Using the Keypad)

Press the Setup Menu button in the middle of the keypad

Use this menu map to navigate the Setup Menu:

PERI	SYR	→	BIOSTK	CARR	PLTCLR
Pump: Prime/Cass/Purge	Manifold type		BioStack	Plate Carrier	Plate Clearance

PERI	SYR	→	BIOSTK	CARR	PLTCLR
Pattern: Row or Column	CAL: Calibration Data		CONF (Mode)	STD/VAC	Offset in mm
Mode: Prompt/Error/Set	Type: Autoclavable /Non-autoclavable/None		ALIGN		

Because variations in the hardware have a big effect on the instrument's performance, you must ensure the instrument's settings match the installed hardware.

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.

Change the Plate Carrier Setting (Keypad)

For LHC instructions see Change the Plate Carrier Setting using the 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. 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
   - MTUBE - Mini-tube carrier.

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.

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).

Using LHC to Control MultiFlo™ Dispenser

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

Predefined Protocols.

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

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

Learn how to Customize the Predefined Protocols on page 59 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 dispenser to the computer.

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

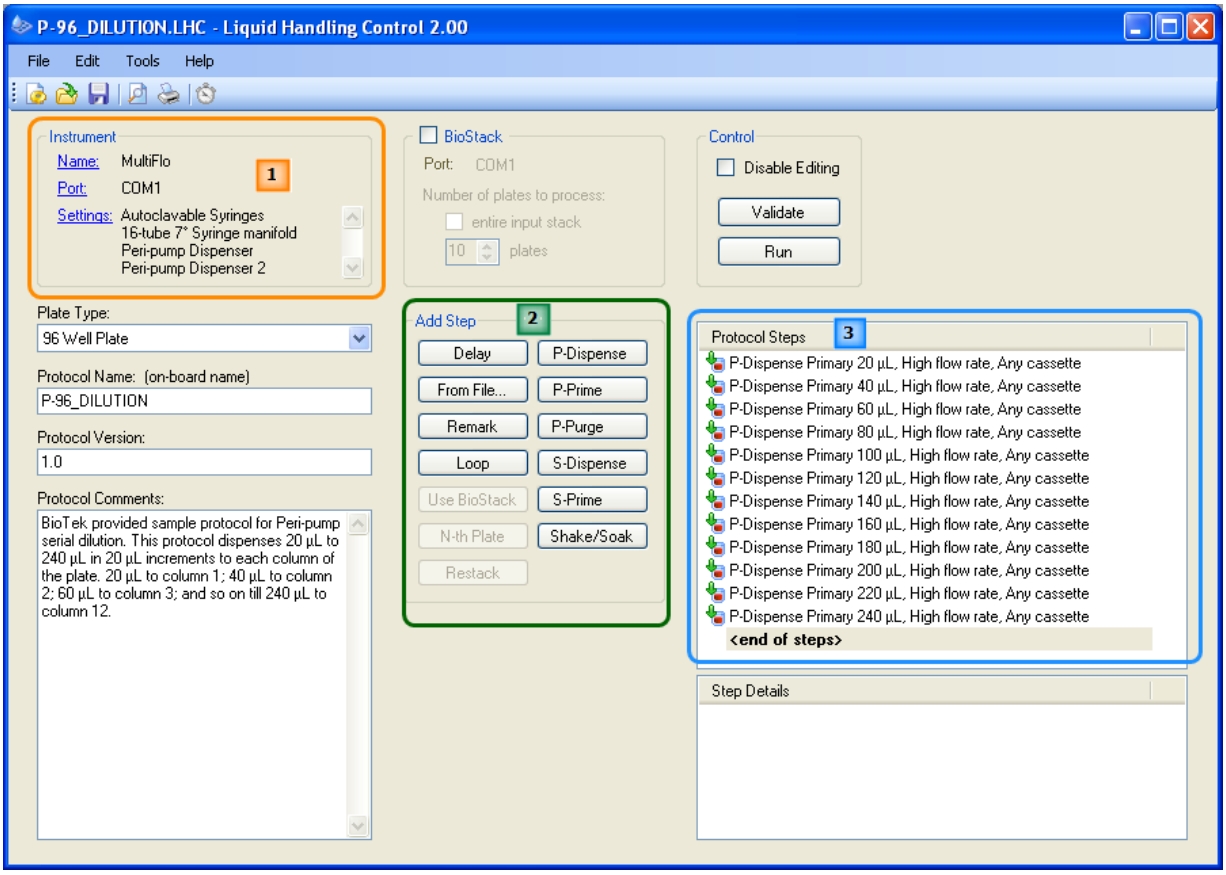
- Learn more **About COM Ports** in the **LHC Installation Guide** or select **Help>Help Topics**.

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 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.

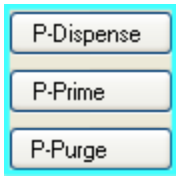
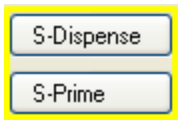
2 MutliFlo Steps: see below.

3 Define a Protocol: select **Help>Help Topics** to learn how.

MultiFlo Steps

Because the MultiFlo offers two dispensers in one instrument: a peristaltic pump dispenser called the Peri-pump, and dual Syringe pump 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:

	**P**- for steps performed by the **Peri-pump** dispenser. One or two Peri-pumps can be installed: **PRI** for primary and **SEC** for secondary.
	**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. Invalid combinations of steps will be identified when you press the **Validate** button.

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 60**

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 59
- **Power Users**: If you create protocols for multiple instruments or for other LHC users, read this more detailed description of how the MultiFlo 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\MultiFlo**

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.

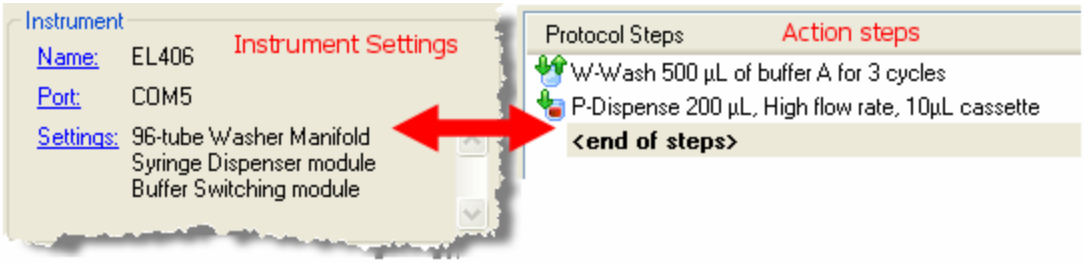
LHC Protocols Explained

Prerequisite

This discussion about MultiFlo 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 MultiFlo 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-

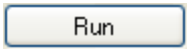
connected instruments, you can create and save an instrument settings file for each of your liquid handlers, another time saver.

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, a MultiFlo 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 MultiFlo 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 MultiFlo to ensure it can successfully perform the 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 MultiFlo 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:

- **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.
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.
  - Optional Second Peri-pump

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 71

Review this topic: Define a Protocol in the LHC's Help for more details. Or, for an in depth description of MultiFlo protocols read this.

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 MultiFlo can process several plate types. The default parameters for dispense steps represent the optimal positioning of the hardware for the plate type. And, the 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 Dispense Processing Patterns on page 93** 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. Only the 32-tube Syringe dispenser manifolds can dispense to 1536-well plates.

Plate Type	On board Name	Columns x Rows	Plate Height	Default Dispense Height	
			mm	Washer	Dispensers
96 Well	96	12x8	14.35	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	120	333
384 Deep Well	384D	24x16	44.08	355	986
384 PCR²	384P	24x16	9.50	83	230
1536 Well	1536	48x32	10.41		250
1536 Flanged‡	153F	48x32	10.26		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 the facing page**

Plate Geometry Diagram

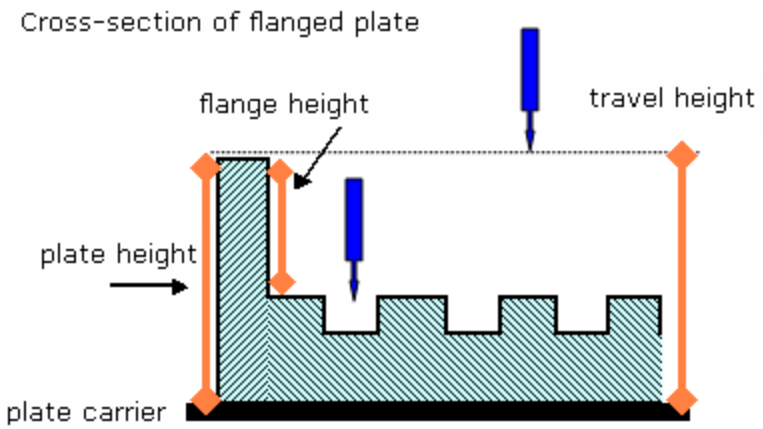


Plate height = physical measurement

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.

Handling Special Plates and Mini-tubes

Note: Also see the Plate Types Table on page 87.

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.

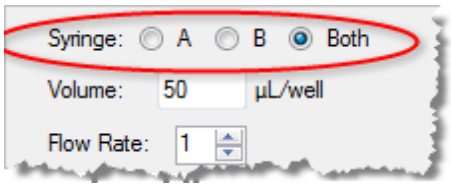
> ▪ Remember, when defining a run: **dispense volume is per tube**, not per well. Divide the desired volume by the number of tubes in a well.

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 151.

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 MultiFlo'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.

Mini-Tube Racks

The MultiFlo supports a special mini-tubes rack, which is available from BioTek as an accessory item:

- 96 Mini-Tubes (96MT), in standard 12 column x 8 row format

Before dispensing to the mini-tubes, install the Special Plate Carrier for Mini-tubes below provided for this vessel. You can use the Peri-pump and 8- and 16-tube Syringe dispensers to fill the mini-tubes.

> ▪ Note: Position adjustments in the Y-axis are prohibited with the special plate carrier for mini-tubes.

Special Plate Carrier for Mini-tubes

Special 24-Vial Rack

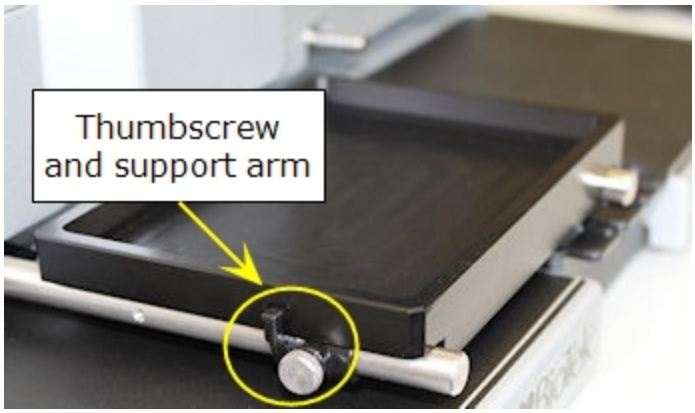
An optional accessory for the MultiFlo, a special plate carrier is needed to support certain special vessels, like a box of mini-tubes. (PN 7212042)

96 Mini Tubes (96MT) is the Plate Type name we give to Corning® 96 Well Cluster Tubes (PN: 4410, 4411).

To dispense to the mini-tubes the special plate carrier must be installed to accommodate the extra height. You can also use the carrier for other oversized vessels that may not otherwise be addressed by the dispensers by increasing the Plate Clearance setting. The maximum height supported with the combination of special carrier and Plate Clearance is about 49-50 mm.

To install the special carrier:

1. Remove the standard carrier: loosen the thumbscrew on the left side of the carrier to release the little support arm and lift the carrier up and off the transport rails.
2. Orient the special carrier so the small cavity for the support arm is on the left, align the hollows under the carrier with the transport rails, and install the carrier.



3. Change the Plate Carrier Setting to match the currently installed plate carrier:
    - Change the Plate Carrier Setting (Keypad) on page 77
    - Change the Plate Carrier Setting (LHC)

Remember to select the proper PLATE in the Quick menu and when creating a protocol.

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.

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.

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.

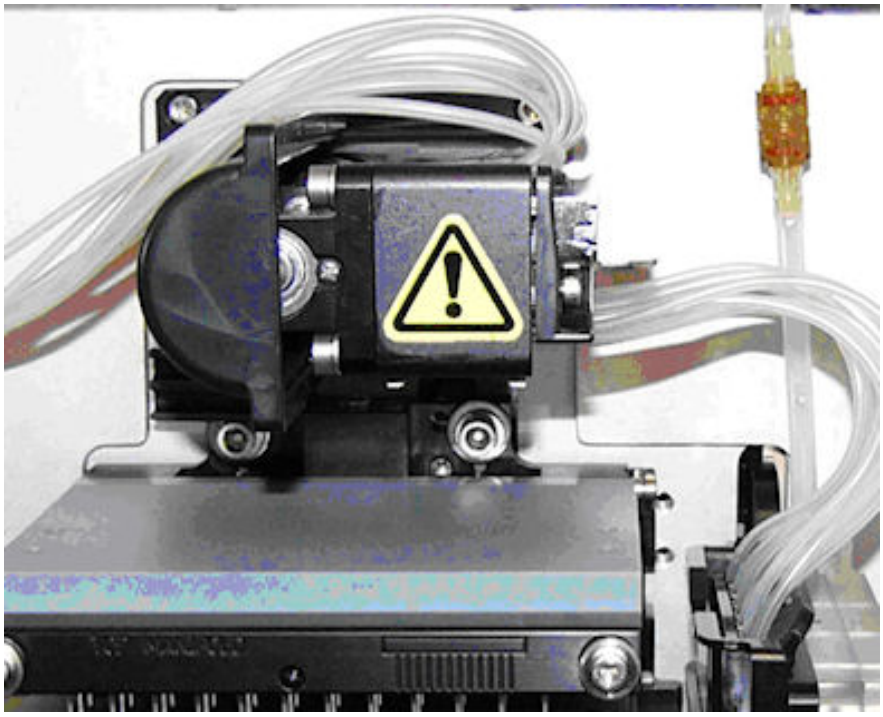
1536-Well Plate

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

	1	2	3	4	5	6	7	8	9	10	11	12
1	1	1	1	1	1	1	1	1	1	1	1	1
2	2	2	2	2	2	2	2	2	2	2	2	2
3	3	3	3	3	3	3	3	3	3	3	3	3
4	4	4	4	4	4	4	4	4	4	4	4	4
5	1	1	1	1	1	1	1	1	1	1	1	1
6	2	2	2	2	2	2	2	2	2	2	2	2
7	3	3	3	3	3	3	3	3	3	3	3	3
8	4	4	4	4	4	4	4	4	4	4	4	4
9	1	1	1	1	1	1	1	1	1	1	1	1
10	2	2	2	2	2	2	2	2	2	2	2	2
11	3	3	3	3	3	3	3	3	3	3	3	3
12	4	4	4	4	4	4	4	4	4	4	4	4
13	1	1	1	1	1	1	1	1	1	1	1	1
14	2	2	2	2	2	2	2	2	2	2	2	2
15	3	3	3	3	3	3	3	3	3	3	3	3
16	4	4	4	4	4	4	4	4	4	4	4	4
17	and so on...											

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.

Peri-pump Peristaltic Dispenser



- Some models do not include a Peri-pump dispenser.

Quick Dispense-Prime-Purge

In addition to controlling the Peri-pump with defined protocol steps, the MultiFlo 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.)

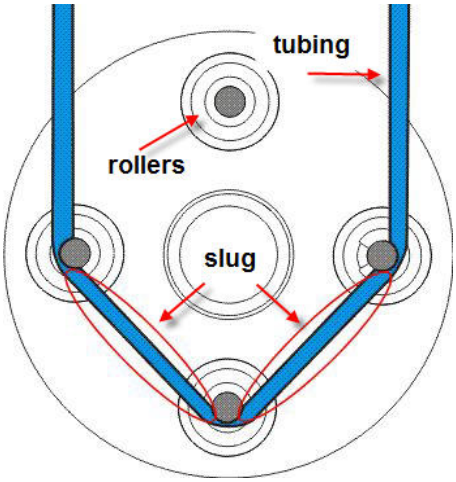
Dispense Cassettes

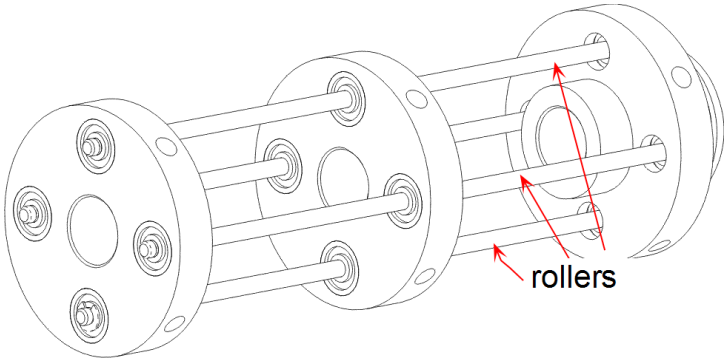
- **Important**: It is imperative that the cassette type setting onboard the instrument match the installed cassette! The cassette type is displayed in the Main Menu and Quick Dispense menu: PRI or SEC-#

- Install the Dispense Cassette on page 27
- Change the cassette type setting on page 29
- Dispense Cassette Diagram on page 26
- Release the tension on the dispense cassette on page 99

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 11**.
- **See Recalibrate the Peri-pump Dispense Cassette on page 151**.

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.

 Use BioTek's Peri-pump Reservoir Holder on page 31 and Shorten the Dispense Cassette Tubing on page 31 to significantly reduce the dead volume to preserve expensive reagents.

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.

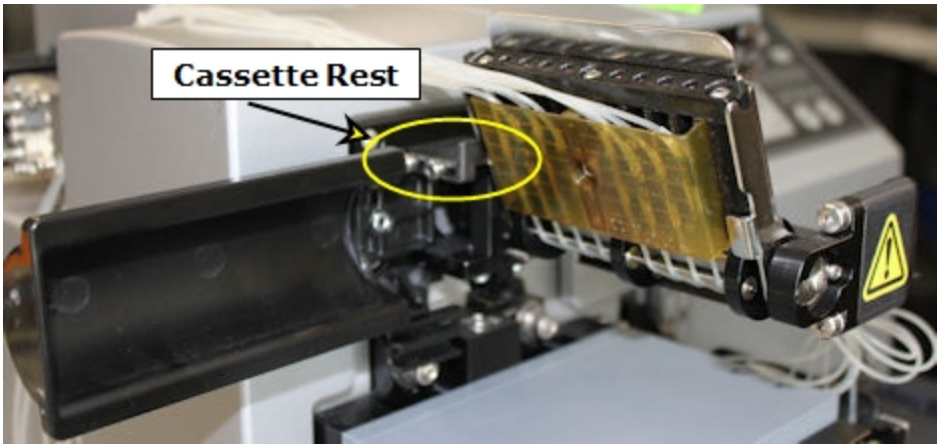
3. Hold the **Prime** button on the keypad:
   - 1 µL cassette = 5 seconds
   - 5 µL cassette = 7 seconds
   - 10 µL cassette = 10 seconds.

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 **PERI**>**DISP** or **HLF-D***

If applicable, select which Peri-pump to use, Primary or Secondary.

* 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 62 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 MultiFlo'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 MultiFlo'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

Settings for 0.5 µL Dispense

LHC: Tools>Instrument Utilities>Peri-pump Dispenser

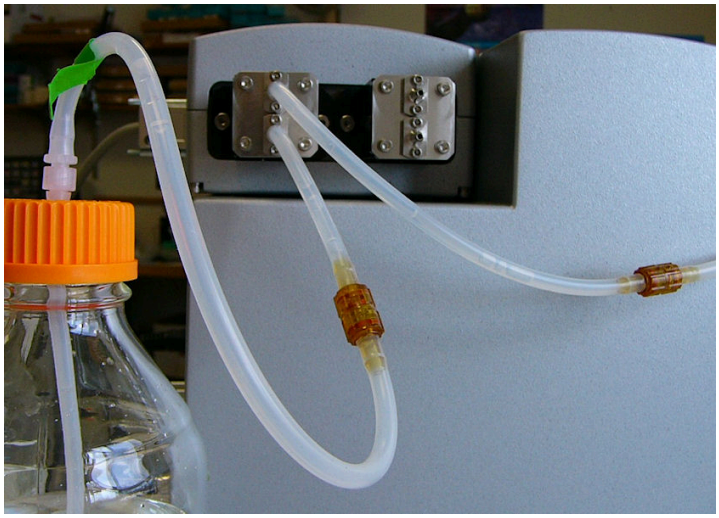
Keypad: Setup Menu>PERI>PUMP>HALF

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 MultiFlo 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 110 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 next page
- See also: Syringe Dispenser Maintenance on page 140

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 step.

Keep the tubing wet during idle time

Turn on AutoPrime for the Syringe Dispenser on page 116

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. Generally, three times the dead volume completely primes the system. 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.

> ▪ **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.

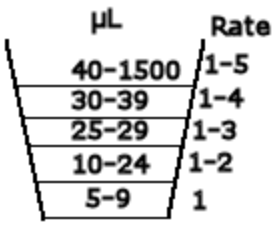
Define the **Flow Rate**: The rate at which the fluid is dispensed. Options range from 1-5, 1 is the slowest, 5 is the fastest. The valid rate is volume dependent. Lower rates are recommended for viscous fluids.

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
µL / Rate 80-3000 / 1-5 60-79 / 1-4 50-59 / 1-3 20-49 / 1-2 10-19 / 1	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.

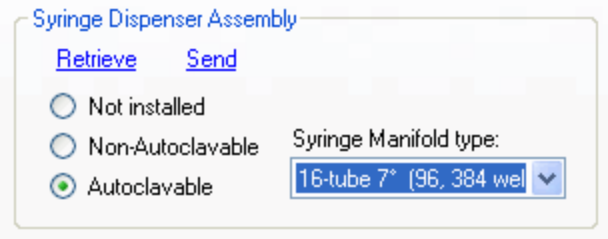
Change the Syringe Dispenser Manifold

Changing the Syringe manifold requires two steps:

- Physically changing the Syringe dispenser manifold: **See Install the Syringe Dispenser Component on page 33**.
- 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
32-tube	1536-well plates only; Model: Large Bore (LB) or Small Bore (SB)

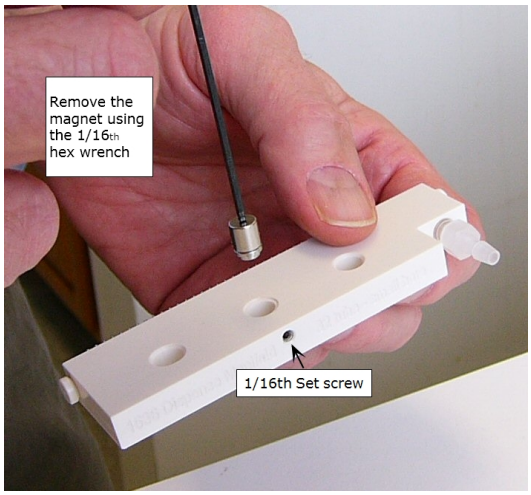
3. LHC: Click **Send** to send this setting to the instrument.
4. Change the Calibration Data to correspond to the installed manifold: if you are changing an 8-tube manifold for a 16-tube manifold, or vice versa, revise the calibration data to match the installed manifold. This step is not necessary when using 32-tube manifolds. Find the labels on the bottom of the Syringe pump module, and enter the corresponding data:

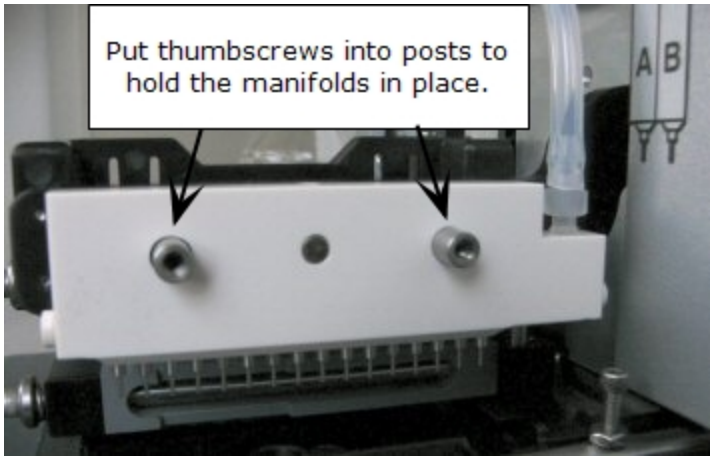
- LHC: Enter the data and click **Send**.
- Keypad: Select **CAL** at the Syringe Setup Menu and enter all the data points by pressing ENTER after each value.

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

Syringe Dispenser Assembly

After installing the Syringe dispenser unit, update the instrument's settings. The MultiFlo automatically detects which type of pump is installed. Select the manifold 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.

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

Look at the top of the manifold to identify its type, which is engraved on the top.

Calibration Data

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.

When installing the Syringe dispenser, enter the values printed on the unit.

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
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)

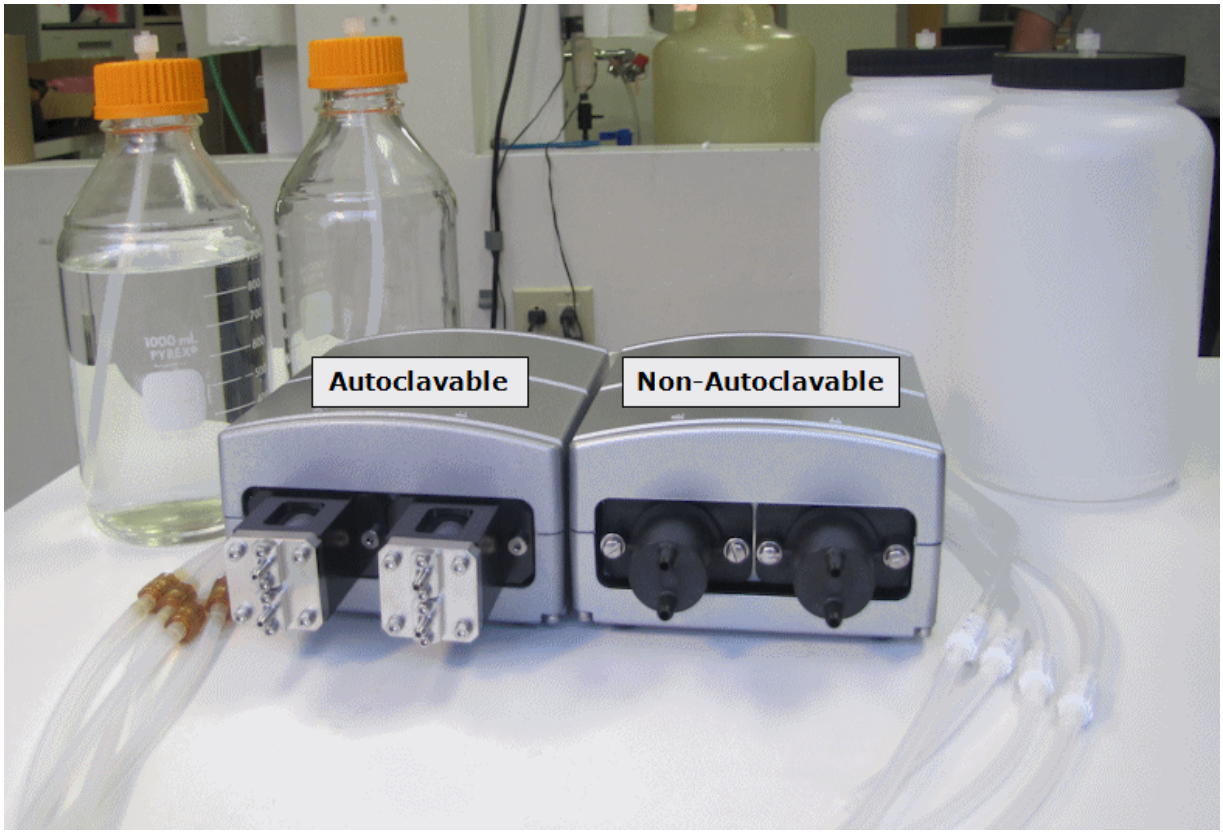
Select CAL to enter (or modify) the calibration data for the Syringes.

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 44

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

Changing the Instrument's Settings

About the Onboard Settings

The instrument's onboard settings dictate its behavior. Certain settings, like Plate Clearance, can improve performance. Other settings are critical to its performance. For example, the manifold setting must match the installed manifold type.

LHC users: do not confuse the LHC's Target Instrument Settings assigned to each protocol with the onboard settings! Review the Help topic: LHC Protocols Explained to learn the distinctions.

To change the onboard settings:

LHC	Keypad
1. Select **Tools>Instrument Utilities** 2. Choose the applicable tab and modify settings as desired. 3. Click **Send** after changing a setting to update the instrument.	1. Press the **Setup Menu** key (in the center of the keypad). 2. Select the applicable device and the setting you want to change. • Alternatively, select **UTILS** at the main menu for AutoPrime, the Adjust Utility and system tests.

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 tubes when addressing the plate. Generally, the components' default positions function perfectly, but certain assays may be improved by repositioning the dispense 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 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
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 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	Options	
Z-axis (up/down)	DISP	Positions the dispense arm.
X-axis (left/right)	CARX	Positions the plate carrier.
Y-axis (front/back)	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.

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 MultiFlo 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 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 MultiFlo 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**;	1. Select **UTIL>AUTPRM**. 2. Set the **AutoPrime Interval**:

LHC	Keypad
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. 5. Click **Send**. Wait for a confirmation message.	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 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.

- **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 MultiFlo 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.

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 (MultiFlo.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 MultiFlo.
3. Press and hold the **Shift** key while restarting the MultiFlo, 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 **MultiFlo.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 MultiFlo 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.

Chapter 4

Maintenance

Properly maintaining the MultiFlo is the key to reliable performance.

Overview

A **Preventive Maintenance (PM)** regimen for the MultiFlo 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 124 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 MultiFlo based on the characteristics of the fluids used and the activity level. Here are some guidelines for each component:

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 142**.

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 next 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
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 dispense tubes				✓	✓
Clean exterior surfaces			✓		
Clean Syringe dispenser manifold				✓	✓
Clean Syringe dispenser tubes				✓	✓
Autoclave Syringe pumps				✓	
Decontaminate					
Decontaminate external surfaces				✓	✓
Run S-DECONTAMINATE				✓	✓
Prepare for Storage or Shipment					
Run S-LONG_SHUTDOWN					✓

Tasks	Frequency				
	Daily	**Overnight/ Multi-Day**	**Weekly**	**Periodic/ Monthly**	**Before storage/ shipment**
Replace/Repair Components					
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 dispensers with an appropriate reagent or deionized water throughout the day. Routine rinsing helps to prevent the 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:

- S-DAY_RINSE_A&B
- P-#UL_CASS_RINSE (# represents the cassette type)(P2 protocols are for the secondary Peri-pump)

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 130.

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 114**.

- 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

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.

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 MultiFlo 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. Turn off the instrument, and lower the manifolds into the priming trough.

- 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 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 **S-Prime** and define parameters to fully prime the tubing, for example:

Syringe Dispenser
Volume = 8000 µL
Flow Rate = 5
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.

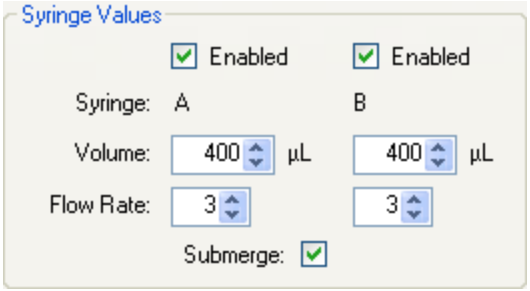
3. Click **Run**. Save the program with a memorable name for future use.

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.

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 MultiFlo 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 MultiFlo'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 MultiFlo 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 dispenser.
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 dispenser.
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 one of the Syringes.
2. Run **S-DECONTAMINATE** protocol, as appropriate.
3. **LHC users**: 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 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 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 the facing page
- Clean the Plate Carrier on the facing page
- Peri-pump Dispenser Maintenance on page 135
- Syringe Dispenser Maintenance on page 140

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:
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 11**.

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.

Clean the Plate Carrier

If liquid has overflowed onto the plate carrier, transport rail, or platform, 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. Loosen the thumbscrew on the left side of the carrier to release the little bracket that holds the carrier in place and remove the carrier.

3. Clean the carrier, rails, and surface, 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:
   - Clip the bottom bracket onto the transport rail, align the little bracket on the left rail to hold the carrier in place and tighten the thumbscrew.
   - If necessary, release the spring-loaded microplate clamp in the back left corner of the carrier to level the carrier on the base.

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 next page
- Unclog the Dispense Tips on page 137
- Record the Number of Plates Processed on page 138
- Recalibrate the Peri-pump Dispense Cassette on page 151
- Replace Peri-pump Dispense Cassette Tubing on page 151

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

Cassette Types	Cassette Life	Total Volume
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.

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.

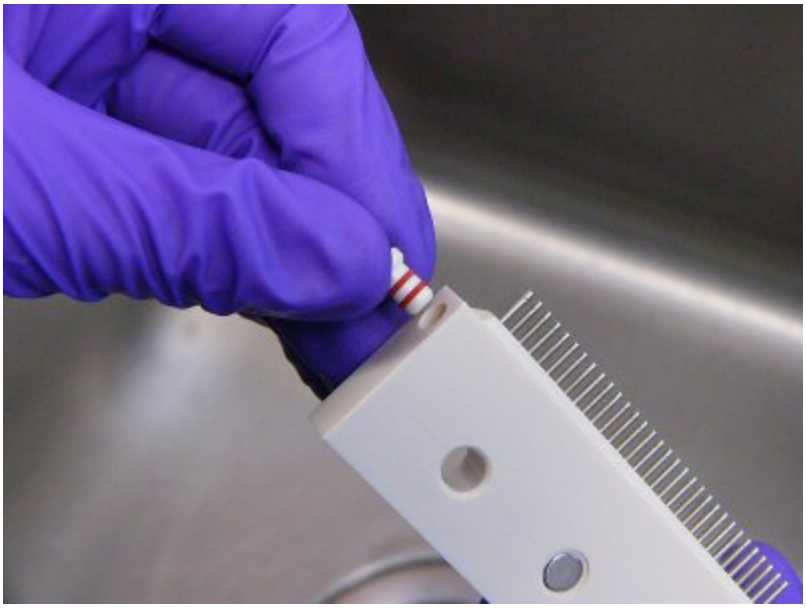
Syringe Dispenser Maintenance Tasks

- Autoclave the Syringe Head on page 143
- Clean the Dispense Tubes on page 142
- Clean the Syringe Dispenser Manifold below
- Clean or Replace the Check Valves on page 143
- 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 next page, if necessary.

Clean the Dispense Tubes

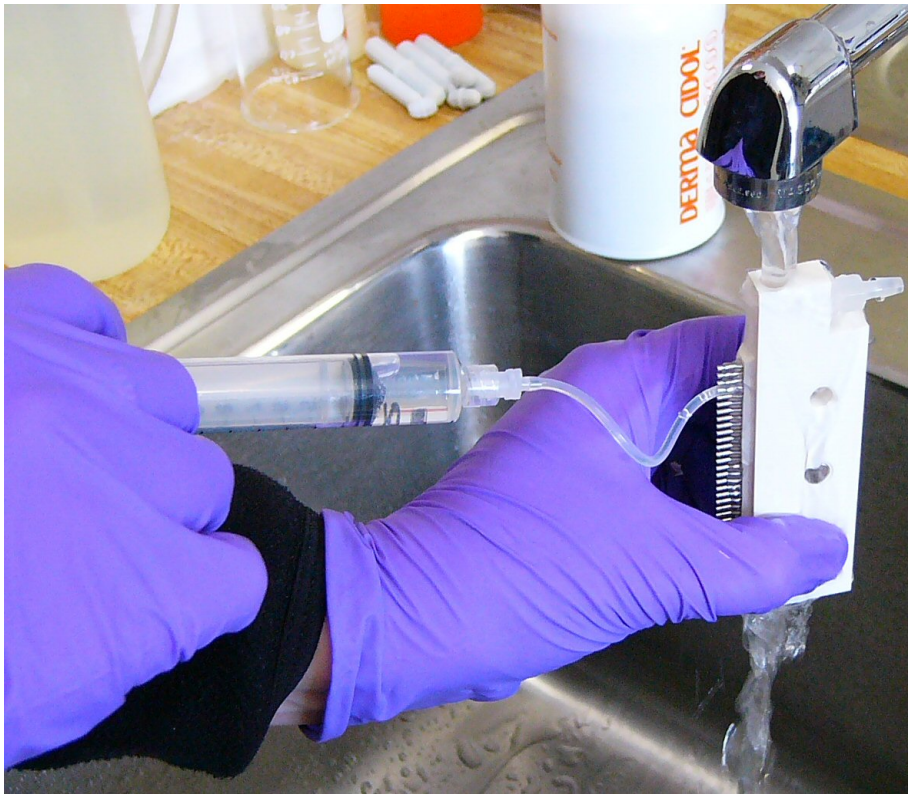
- **Do NOT autoclave the dual 8-tube and 32-tube dispense manifolds!**

- 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 175.

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.

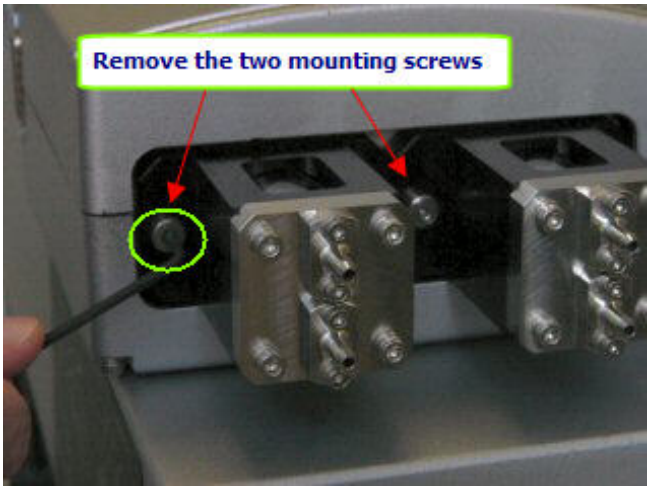
To clean a check valve:

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.

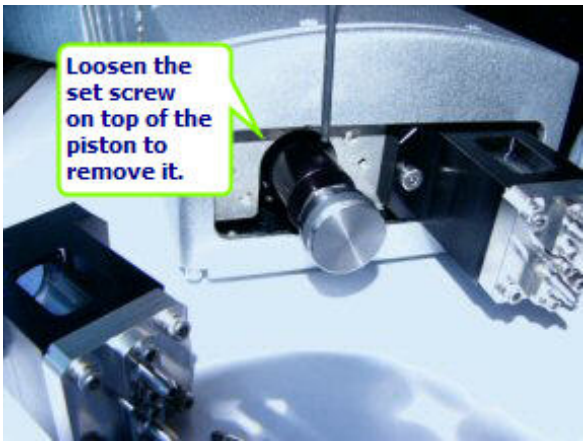
Replace Syringe Dispenser Check Valve on page 152, if necessary.

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 110**.



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 149

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.
   - 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 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:

- **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).
5. Reconnect the power cord and turn on the instrument.
6. Run the decontamination protocols.

Preparing to run S-DECON or S-DECONTAMINATE:

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.

Long Shutdown (Prepare for Storage or Shipment)

Before the MultiFlo 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:

- **S-LONG_SHUTDOWN** — for the Syringe dispenser

The LONG_SHUTDOWN protocol flushes and soaks the supply and manifold tubing with disinfectant, then flushes with rinse, and finally purges 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).
8. Turn on the instrument and run **S-LONG_SHUTDOWN**.

Storing the Instrument

After performing the Long Shutdown (Prep for Storage or Shipment) above 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 MultiFlo must be replaced periodically to maintain specified performance levels.

Peri-pump Components

- Replace Peri-pump Dispense Cassette Tubing below
- Recalibrate the Peri-pump Dispense Cassette below

Syringe Dispenser Components

- Clean or Replace the Check Valves on page 143
- Calibrate the Backlash for Syringe Dispenser on page 153

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 MultiFlo 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 MultiFlo 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 MultiFlo 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 the facing page.

Calibrate the Backlash for Syringe Dispenser

If you have replaced a check valve 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

Instrument verification for the MultiFlo 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 the facing page.

Verification testing includes:

- The **System Self Test** verifies system components, such as the manifold and carrier positioning. The **Checksum Test** verifies the basecode software against internal checksum values to ensure that no corruption has occurred.

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 164

Syringe Dispenser Qualification Tests:

- Perform the Syringe Dispense Precision & Accuracy Test on page 175

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 MultiFlo is properly maintained as outlined in the Recommended Maintenance Schedule on page 124.

- **Note:** An instrument qualification package (PN 7210512) 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	✓		
System Self Test and Checksum Test	✓	✓	✓
Shake 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.

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 MultiFlo 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 MultiFlo.

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**	1. Select **UTILS** at the main menu.

Utilities>Software.

2. Click the **Retrieve All** onboard settings link at the top of the window.

2. Select **TESTS**.
3. Select **CHKSUM**.
4. One at time, display the **UI** and **MC** checksums.

Liquid Testing the MultiFlo™ Microplate Dispenser

Which Tests to Perform?

We recommend that you perform these routine tests before first use (after the IQ) and then monthly:

Peri-pump Dispenser

- **Dispense Precision and Accuracy Test.** For each Peri-pump installed 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.

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 159**.

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 MultiFlo 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** (P2 protocols are designed for the Secondary Peri-pump).

- 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 137). 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 the facing page.

> ▪ See also **Important Recommendations for All Liquid Tests on page 159.**

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.

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 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 MultiFlo, 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%
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

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 MultiFlo, 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 201 (6000-6100) - protocol errors
- System Error Codes on page 194 (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 MultiFlo 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.

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

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 electrical problem.	Turn instrument off, wait at least 15 seconds, turn 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 MultiFlo'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 57
	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 41**.
	Check valves are stuck closed.	Clean or Replace the Check Valves on page 143
	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	Clogged dispense tubes on	Clean the Syringe Dispenser

Problem	Possible Cause	What To Do
fluid; wells not filled.	the dispenser manifold.	Manifold on page 140
	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 143
	32-tube dispense manifolds are not dispensing accurately.	Calibrate the Backlash for Syringe Dispenser on page 153
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-tube SB manifold.	Minute particles of debris on the end of the tubes.	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.

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 143
	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 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.

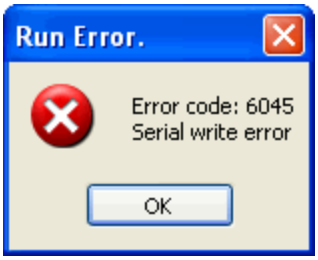
Peri-pump Troubleshooting

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 columns	Check/define the Plate Map.
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 MultiFlo 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.



BioStack communication error: when controlling the BioStack with the LHC, make sure the onboard configuration setting is **Manual**.

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 MultiFlo 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 MultiFlo 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

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	Secondary Peri-pump 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.
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	Secondary Peri-pump 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	Secondary Peri-pump motor failed positional verify	Service Only. Contact BioTek TAC.
404	Syringe A motor failed positional verify	Service Only. Contact BioTek TAC.
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.

Code	Message	What to do
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.
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.
1300	Invalid syringe selection	Contact BioTek TAC.

Code	Message	What to do
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.
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.
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.

Code	Message	What to do
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.
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.
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.
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.
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.

Code	Message	What to do
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.
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.

Code	Message	What to do
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.

MultiFlo-LHC 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.

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 MultiFlo 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 191
6001	COM port created by USB converter no longer active	See *Communication Port*
6002	Invalid basecode part number; instrument is not an MultiFlo	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 match the currently installed hardware.

Error Code	Description	Help
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**.

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