

MicroFlo™ Select

Operator's Manual



MicroFlo Select™

**Microplate Dispenser
Operator's Manual**

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Part Number 7171000
Revision C
BioTek® Instruments, Inc.

Notices

BioTek® Instruments, Inc.
Highland Park, P.O. Box 998
Winooski, Vermont 05404-0998 USA

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Contacting BioTek Instruments, Inc.

BioTek® Instruments, Inc.
Highland Park, P.O. Box 998
Winooski, Vermont 05404-0998 USA

Customer Service and Sales

Internet: www.biotek.com
Phone: 888-451-5171 (toll free in the U.S.)
802-655-4740 (outside the U.S.)
Fax: 802-655-7941
E-Mail: customercare@biotek.com

Service/TAC

Phone: 800-242-4685 (toll free in the U.S.)
802-655-4740 (outside the U.S.)
Fax: 802-654-0638
E-Mail: tac@biotek.com

European Coordination Center/ Authorized European Representative

BioTek® Instruments GmbH
Kocherwaldstrasse 34
D-74177 Bad Friedrichshall
Germany
Internet: www.biotek.de
Phone: +49 (0) 7136 9680
Fax: +49 (0) 7136 968 111
E-Mail: info@biotek.de


Revision History


| Rev | Date | Changes |
|-----|---------|---|
| A | 10/2007 | First Issue |
| B | 08/2008 | Revised the repackaging artwork and instructions in the Installation chapter to reflect new packaging materials. Removed the hyphen from BioStack. Updated the Trademarks section by adding the BioStack, Liquid Handling Control™ Software, Microsoft, and Excel. Added a Cassette Warranty to the Preface. Updated the Registration section in the Preface by adding an instruction to review the Warranty for the instrument and register online through the Customer Resource Center. Updated the TAC fax number and added "ATTN: RMA # xxxxx" to the BioTek shipping address (for returning the instrument). Added a part number disclaimer in the Package Contents and Optional Accessories sections. |
| C | 02/2010 | Description and pictures of the Dispense Cassette were revised to match new design. Added descriptions of the Tip Guard and break-away tabs. Added description of the new Plate Clearance Height parameter. Optional accessories were updated with angled dispensing and tubing extensions products. Revised the Intended Use statement for IVD. |

Document Conventions

The intent of this operator's manual is to instruct the new user how to set up and operate BioTek's MicroFlo Select Microplate Dispenser. To help you read and understand this manual, certain document conventions have been used.

This manual uses the following typographic conventions:


| Example | Description |
|---|---|
|  | This icon calls attention to important safety notes. |
| Warning! | A Warning indicates the potential for bodily harm and tells you how to avoid the problem. |
| Caution | A Caution indicates potential damage to the instrument and tells you how to avoid the problem. |

| Example | Description |
|---|---|
|  | This icon calls attention to important information. |

The menu path to open the feature described is shown in this format.

When applicable, the above format shows you how to navigate to the feature described in that section.

❖ **Note:** Information of particular importance or usefulness is presented in this format.

 Tips or other helpful information is presented in this format.

Intended Use Statement

BioTek's MicroFlo Select™ Microplate Dispenser accommodates all standard microplates and deep well blocks for bulk reagent dispensing applications. The dispense tubing cassettes are autoclavable for those applications requiring a sterile fluid path.

The MicroFlo Select™ can operate with standard robotic systems, such as BioTek's BioStack™ Microplate Stacker.

This product may be used for In Vitro Diagnostic, research and development, or other 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 or standard laboratory protocol for the test to be conducted. Failure to conduct Quality Control checks could result in erroneous test data.

Regulatory Agency Approvals

The MicroFlo Select Microplate Dispenser has been tested, approved, and labeled to show compliance to the test standards indicated on later pages in this section.

Warranty & 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.

Cassette Warranty

MicroFlo Select dispense cassettes are backed by BioTek's **Confidence-Plus** lifetime warranty. For the lifetime of the cassette, BioTek guarantees the cassette components (Tip Holder, Center Holder, Tube Tensioner, and Tube Organizer) will withstand steam autoclave conditions of 121°C and 1 bar (750 mmHg) without adversely affecting dispense performance. In the event of a failure of any cassette component previously listed, BioTek will replace the cassette components free of charge.

Warnings



Operate the instrument on a flat surface away from excessive humidity.

When operated in a safe environment according to the instructions in this document, there are no known hazards associated with the instrument. However, the operator should be aware of certain situations that could result in serious injury; these vary depending on the instrument type. See **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.



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.**

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 for more information and 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 listed range of line voltages.

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 must be removed before operating the instrument and reinstalled before repackaging the instrument for shipment.

Caution: Do not run the MicroFlo Select 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.

CE Mark



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

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 and EN 61326-2-6: 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 61326-1:2003 and CISPR 16-2:1999 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

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

Directive 73/23/EEC: Low Voltage (Safety)

The system has been type tested by an independent testing laboratory and was found to meet the requirements of EC Directive 73/23/EEC for Low Voltage. Verification of compliance was conducted to the limits and methods of the following:

EN 61010-1 (2001) 2nd Edition

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

EN 61010-2-81 (2003)

“Requirements for automatic and semi-automatic laboratory equipment for analysis and other purposes.”

EN 61010-101 (2002)

“Particular requirements for in vitro diagnostic (IVD) medical equipment”

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 9.

Directive 98/79/EC: In Vitro Diagnostics

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

Electromagnetic Interference and Susceptibility

USA FCC CLASS A

Warning: Changes or modifications to this unit not expressly approved by the manufacturer could void the user's authority to operate the equipment.

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. Like all similar equipment, 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 interference, in which case the user will be required to correct the interference at their own expense.

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'emet pas du 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: 2004**

“Safety requirements for electrical equipment for measurement, control and laboratory use; Part 1: general requirements”









- **Canadian Standards Association CAN/CSA C22.2 No. 61010-1-04**

“Safety requirements for electrical equipment for measurement, control and laboratory use; Part 1: general requirements”

- **EN 61010 Standards – See CE Mark List**

Safety Symbols

Some of these symbols appear on the instrument or accessories:

| | |
|---|---|
|  <p>Alternating current Courant alternatif Wechselstrom Corriente alterna Corrente alternata</p> |  <p>Both direct and alternating current Courant continu et courant alternatif Gleich - und Wechselstrom Corriente continua y corriente alterna Corrente continua e corrente alternata</p> |
|  <p>Direct current Courant continu Gleichstrom Corriente continua Corrente continua</p> |  <p>Earth ground terminal Borne de terre Erde (Betriebserde) Borne de tierra Terra (di funzionamento)</p> |
|  <p>On (Supply) Marche (alimentation) Ein (Verbindung mit dem Netz) Conectado Chiuso</p> |  <p>Protective conductor terminal Borne de terre de protection Schutzleiteranschluss Borne de tierra de protección Terra di protezione</p> |
|  <p>Off (Supply) Arrêt (alimentation) Aus (Trennung vom Netz) Desconectado Aperto (sconnessione dalla rete di alimentazione)</p> |  <p>Caution (refer to accompanying documents) Attention (voir documents d'accompagnement) Achtung siehe Begleitpapiere Atención (vease los documentos incluidos) Attenzione, consultare la doc annessa</p> |

| | | | |
|---|--|--|---|
|  | <p>Warning, risk of electric shock Attention, risque de choc électrique Gefährliche elektrische schlag Precaución, riesgo de sacudida eléctrica Attenzione, rischio di scossa elettrica</p> |   | <p>Warning, risk of crushing or pinching Attention, risque d'écrasement et pincement Warnen, Gefahr des Zerquetschens und Klemmen Precaución, riesgo del machacamiento y sejeción Attenzione, rischio di schiacciare ed intrappolarsi</p> |
|  | <p>Warning, hot surface Attention, surface chaude Warnen, heiße Oberfläche Precaución, superficie caliente Attenzione, superficie calda</p> |  | <p>Warning, potential biohazards Attention, risques biologiques potentiels Warnung! Moegliche biologische Giftstoffe Atención, riesgos biológicos Attenzione, rischio biologico</p> |
|  | <p>In vitro diagnostic medical device Dispositif médical de diagnostic in vitro Medizinisches In-Vitro-Diagnostikum Dispositivo médico de diagnóstico in vitro Dispositivo medico diagnostico in vitro</p> |  | <p>Separate collection for electrical and electronic equipment Les équipements électriques et électroniques font l'objet d'une collecte sélective Getrennte Sammlung von Elektro- und Elektronikgeräten Recogida selectiva de aparatos eléctricos y electrónicos Raccolta separata delle apparecchiature elettriche ed elettroniche</p> |
|  | <p>Consult instructions for use Consulter la notice d'emploi Gebrauchsanweisung beachten Consultar las instrucciones de uso Consultare le istruzioni per uso</p> | | |

Chapter 1

Introduction

This chapter introduces the MicroFlo Select Microplate Dispenser, and describes its hardware and software features.

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The MicroFlo Select Microplate Dispenser

The MicroFlo Select (MicroFlo) is a self-contained, user-programmable, *universal* microplate dispenser. The following unique combination of features expands applications far beyond the typical microplate 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 which 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 9999 μL .
- The tubing is autoclavable (steam temperatures and pressures of 121° C and 1 bar (750 mmHg)) and compatible with 70% ethyl or isopropyl alcohol and 0.5% sodium hypochlorite (bleach) solution.
- The dispenser's standard carrier accommodates 6-, 12-, 24-, 48-, 96-, 384-, and 1536-well plates that comply with SBS microplate standards 4-2004. A custom carrier is available for dispensing into 12 mm X 75 mm and 13 mm X 100 mm test tubes. Plate types are stored in the onboard software.
- Dispensing can be performed instantly for the most common applications. The MicroFlo saves the 10 most-recently-used dispense routines for quick access. Dispense volumes, plate type, and plate maps can be defined on-the-fly.
- User-programmable dispense rates provide for a wide range of dispensing capabilities.
- The horizontal dispense position provides for precise positioning of the wells under the dispense tubes.
- A variety of solutions, including buffered saline and various reagents, can be dispensed.
- The intuitive menu-driven software allows you to create and store up to 99 dispense protocols.
- The MicroFlo has a low-maintenance design, the result of BioTek's long history with liquid-handling instruments.
- In addition to operating on a stand-alone basis, the MicroFlo is compatible with BioTek's BioStack™ Microplate Stacker and BioStack Twister II Microplate Handler.

Software

The MicroFlo dispenser is operated by onboard software, controlled through the dispenser's keypad or with BioTek's Liquid Handling Control (LHC) Software, purchased separately.

The onboard software, called the "basecode," has an identifying **part number: 7170200** and **version number**.

The **7170200** basecode supports the following features:

- An easy-to-use, menu-driven interface.
- One-button launch of Prime, Purge, and Quick Dispense programs.
- Storage of up to 99 programs.
- User-definable program names, to easily distinguish them at runtime.
- Configurable program parameters such as variable dispense flow rates, dispense heights, and plate map, i.e., the columns to dispense to.
- Optionally, the LHC provides computer control of the MicroFlo and the MicroFlo Select Interface Software offers a .NET software component for robotics programmers.

Compatibility with the BioStack Microplate Stacker

The MicroFlo Select is compatible with BioTek's BioStack Microplate Stacker. Controlled serially by the MicroFlo or BioTek's Liquid Handling Control (LHC) software, the BioStack system can rapidly and systematically transfer microplates one at a time, to and from the dispenser, and includes:

- Removable stacks (one input and one output) that can hold up to 30 microplates each.
- Optional restacking of plates to maintain correct sequencing.
- The ability to continue processing a stack of plates following the aborting/failure of one plate.
- The ability to pause processing 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 MicroFlo, refer to the ***BioStack Operator's Manual*** for instructions on configuring the dispenser to run with the BioStack. If you are interested in purchasing the BioStack, contact your local BioTek dealer for more information.

Hardware Features

- 2-line x 24-character LCD display.
- Membrane keypad with 25 alphanumeric keys.
- External 24-volt power-supply is compatible with 100 - 240 V~; 50 - 60 Hz.
- One serial RS-232 COM port (9-pin female connector).
- One USB COM port.
- Two user-selected tubing cassettes.
- Controllable flow rates and volumes provided by a peristaltic pump.
- Stepper motors provide accurate and repeatable carrier and dispense head positioning.
- Plate Carrier and Priming Trough are easily removed for cleaning.
- Prime Trough Insert is supplied as an accessory to capture expensive reagents for reuse.

Package Contents

The contents of the MicroFlo Select Microplate Dispenser package include:

| Part | Part Number |
|---|--------------------------|
| Dispenser | MFS or MFS1536 |
| Fluid vessel and holder | 7090564 |
| Power cord | varies by country of use |
| Power supply | 61062 |
| USB Cable | 75108 |
| 12 X 8 strip microplate | 98265 |
| Priming trough insert | 7172062 |
| Priming trough drain hose | 67483 |
| MicroFlo™ Select Installation Guide | 71710013 |
| MicroFlo Select Operator's Manual/User Support Tools on CD | 7171014 |
| Tubing Cassettes (2) | Customer specified |
| Small plastic sealable bag containing <ul style="list-style-type: none"> • a screwdriver, • hex wrench, • 10 cc syringe, and a self-adhesive strip for securing the bag to the back of the instrument. | |

❖ Part numbers are subject to change over time. Please contact BioTek Customer Care if you have any questions.

Optional Accessories

| Part | Part Number |
|---|-------------|
| Liquid Handling Control™ Software | LHC |
| MicroFlo Select Interface Software | 7170202 |
| MicroFlo Select Product Qualification Package | 7170521 |
| Test tube rack | 7170520 |
| Cassette Calibration Kit | 7170017 |
| Angled Dispensing Kit | 7180010 |

Dispense cassettes and accessories:

| Cassette Type | PN | Tips | Replacement tubing kit | Tubing extension kit |
|-----------------------|---------|---------|------------------------|----------------------|
| 1 µL molded tip | 7170012 | 7172060 | 7170009 | 7170022 |
| 1 µL 1536 molded tips | 7170018 | | | |
| 1 µL steel tip | 7170015 | 7172129 | 7170008 | 7170021 |
| 1 µL 1536 steel tips | 7170016 | | | |
| 5 µL molded tip | 7170011 | 7172059 | 7170007 | 7170020 |
| 5 µL steel tip | 7170014 | 7172128 | | |
| 10 µL molded tip | 7170010 | 7172039 | 7170007 | 7170020 |
| 10 µL steel tip | 7170013 | 7172127 | | |

❖ Part numbers are subject to change over time. Please contact BioTek Customer Care if you have any questions.

Specifications

Labware

| | | |
|-------------|--|---|
| Microplates | 96-, 384-, 1536-well | Standard, low profile, or deep-well depth with outside dimensions of 5.030" ± 0.010" x 3.365" ± 0.010" (127.76 mm ± 0.25 mm x 85.47 mm ± 0.25 mm) |
| | 6-, 12-, 24-, 48-well | |
| Microstrips | 1 x 8, 1 x 12 | Standard |
| Microwells | Flat, round, "V" bottom, PCR, or half-area | |
| Test tubes | 12 mm X 75 mm | Test tube rack accessory available from BioTek is required (PN 7170520) |
| | 13 mm X 100 mm | |

Hardware & Environmental

| Cassette Types | Dispense range | Cassette Life | Dead Volume | |
|----------------|--------------------------------|----------------------------------|-------------|------------|
| | | | 24" tubing | 30" tubing |
| 1 µL | 1 – 50µL in 1µL increments | 1000 384-well plates @ 5µL/well | .96 mL | 1.2 mL |
| 1 µL 1536 | 1 – 50µL in 1µL increments | 250 1536-well plates @ 5µL/well | .96 mL | 1.2 mL |
| 5 µL | 5 – 2500µL in 5µL increments | 1000 96-well plates @ 50µL/well | 3.4 mL | 4.2 mL |
| 10 µL | 10µL – 10mL in 10µL increments | 1000 96-well plates @ 100µL/well | 5.9 mL | 7.4 mL |

| | |
|-----------------------|--|
| Peristaltic pump: | Positive displacement peristaltic pump with 4 rollers that stretch the 8 tubes (one per channel) to deliver fluid |
| User Interface: | 2-line x 24 character LCD screen 25 alphanumeric soft keys |
| Power Supply: | The instrument uses a 24-volt 90 watt external power supply that is compatible with 100-240 V~; 50-60 Hz. |
| Dimensions: | 14x10.3x11.5 inches (36x26x29.2 cm) (WxDxH), |
| Weight: | 15 lbs. (6.67 kg) |
| Operating Conditions: | 10° - 40°C (50° - 104°F) |
| Relative Humidity: | The instrument should be operated in a noncondensing humid environment having a maximum relative humidity of 80% at temperatures up to 31° decreasing linearly to 50% relative humidity at 40°C. |

Dispense Precision and Accuracy

| Cassette | Precision | Accuracy |
|----------------|---------------------------------|---------------------------------|
| 1 μ L | < 10%CV @ 1 μ L per well | \pm 10% @ 1 μ L per well |
| | < 5%CV @ 2 μ L per well* | \pm 5% @ 2 μ L per well* |
| 1 μ L 1536 | < 10%CV @ 1 μ L per well | \pm 10% @ 1 μ L per well |
| | < 5%CV @ 2 μ L per well* | \pm 5% @ 2 μ L per well* |
| 5 μ L | < 5%CV @ 5 μ L per well | \pm 4% @ 5 μ L per well |
| | < 2.5%CV @ 10 μ L per well* | \pm 2% @ 10 μ L per well* |
| 10 μ L | < 4%CV @ 10 μ L per well | \pm 4% @ 10 μ L per well |
| | < 2%CV @ 20 μ L per well* | \pm 2% @ 20 μ L per well* |

❖ * These specifications are for these dispense volumes and higher.

Chapter 2

Installation

This chapter includes instructions for setting up the MicroFlo Select Microplate Dispenser and installing its components. Instructions for repackaging the instrument for shipment are also included.

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1: Unpack and Inspect the Instrument



Important! Save all packaging materials. If you need to ship the instrument to BioTek for repair or replacement, you must use the original packing materials. Using other forms of commercially available packing materials, or failing to follow the repackaging instructions, may void your warranty.

If the original packing materials have been damaged, replacements are available from BioTek.

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

If the MicroFlo Select 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.

Accessories Checklist

Use this checklist to make sure you have all the accessories:

- 2 Tubing cassettes (customer specified)
- Reagent bottle holder
- Strip plate 12 x 8
- Priming trough insert
- Prime trough drain tube
- Installation instructions and User's Guide on CD
- Power supply and power cord
- USB cable
- Small plastic pouch

- Screw driver for calibrating cassettes
- 10 cc syringe and tubing for flushing cassette tubes and tips
- Hex wrench for removing shipping hardware
- Velcro® strips for attaching plastic pouch on rear panel

Optional accessories shipped separately:

- Liquid Handling Control™ (LHC) software
- MicroFlo Select Qualification and Maintenance Package
- Cassette Calibration kit
- Carrier test tube rack
- Angled Dispense Kit

2. Remove the shipping hardware

Two shipping brackets and a rubber band protect the MicroFlo during shipping. After removing the dispenser from its boxes and bag, place it on a level work surface to remove the shipping hardware.

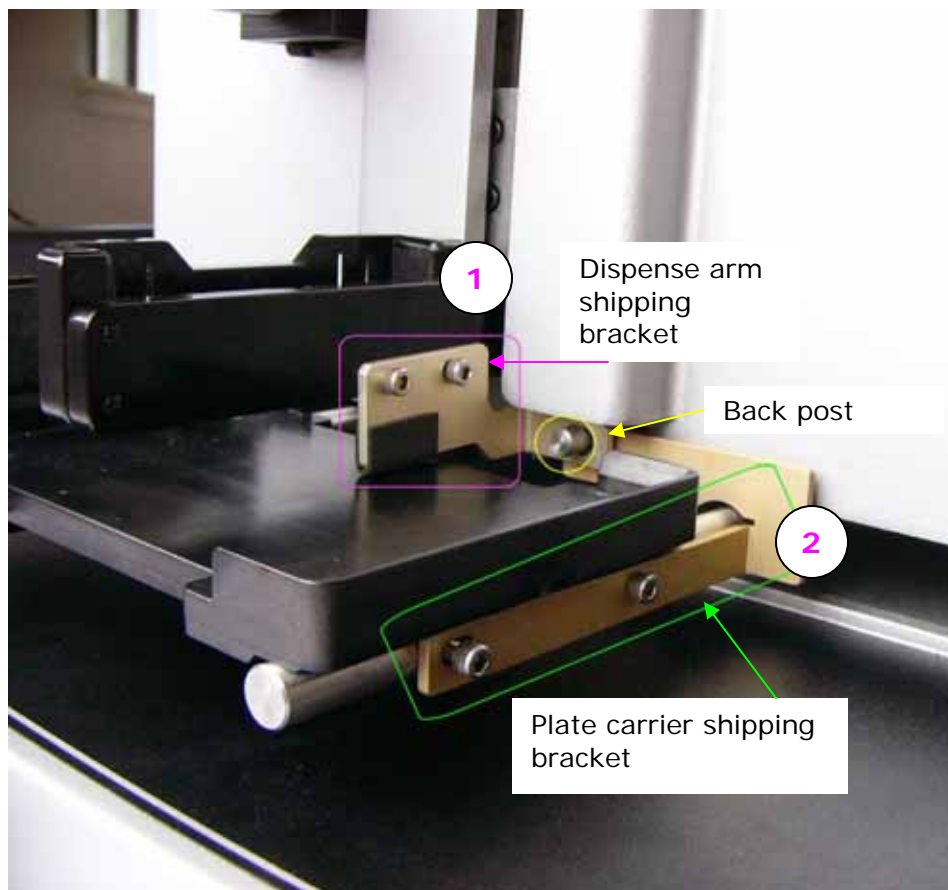


Figure 1: Shipping Hardware

Required Materials

- Hex wrench shipped in small plastic bag

Remove the shipping brackets

1. First remove the dispense arm shipping bracket: use the hex wrench to remove the two screws that hold it to the dispense arm and slide it forward and off the back rail.
2. Next, remove the plate carrier shipping bracket: use the hex wrench to remove the two screws that hold it to the plate carrier and slide it forward and off the back rail.
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.

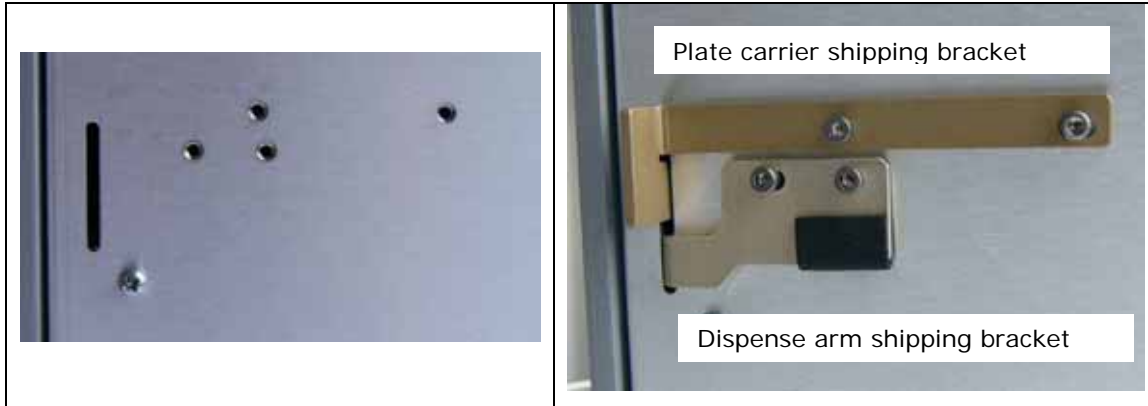


Figure 2: Attach the shipping brackets to the back of the instrument for safe keeping.

Four screw holes and a slot on the rear panel of the dispenser, (left side, near the top), are provided for storing the shipping brackets. Line up the brackets' screw holes with the holes in the back and insert the brackets' arms into the slot. Then, use the shipping screws to attach the brackets to the instrument.

Store the hex wrench in the plastic storage pouch.

Remove the rubber band

4. Carefully remove the rubber band that holds the pump cover in place around the pump.

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

Attach the plastic storage pouch to the rear panel

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

Now, you're ready to complete the installation by installing its components.

3: Install the MicroFlo's Components

With the shipping hardware removed, you're ready to install:

- Tubing Cassette
- Prime Trough Drain Tube
- (Optional) Prime Trough Insert
- Fluid supply vessel
- Power Cord
- (Optional) Serial or USB Cable for communication with computer and/or BioStack™ Microplate Stacker.

These components are all that is required to run the MicroFlo to process standard microplates. Additional steps are required to dispense to test tubes and plates with fewer than 96 wells. Review the section on **Special Plates** in the *Essential Concepts* chapter.

Install the Tubing Cassette

Take a moment to identify the MicroFlo's parts that are named in the instructions for installing the tubing cassette.

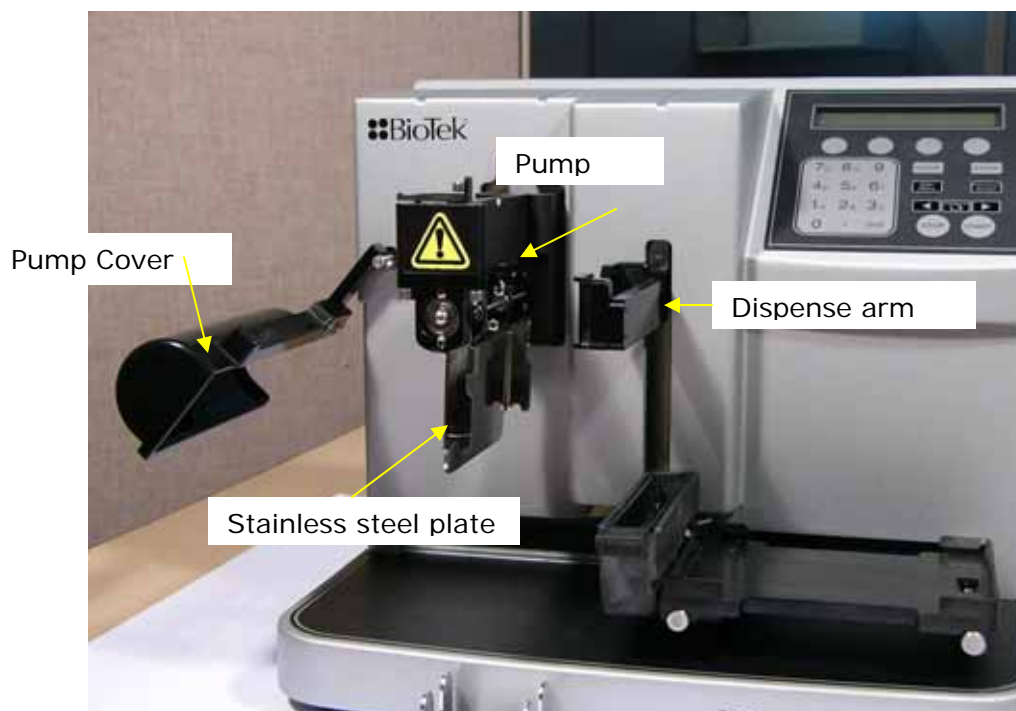


Figure 3: Dispenser ready for cassette installation.

❖ The Pump Cover must be closed to operate the dispenser.

Tubing Cassette Diagram

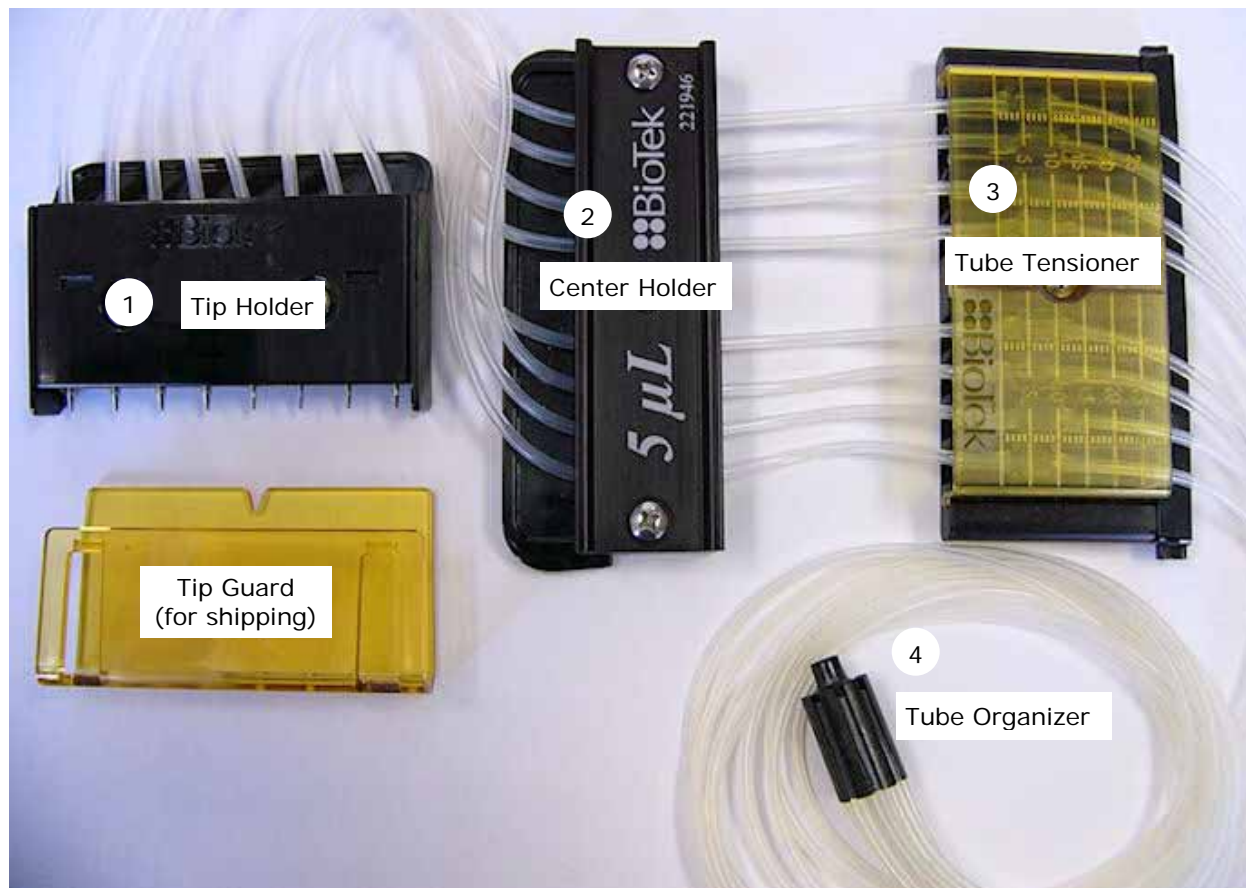


Figure 4: Cassette Diagram

❖ **Important:** remove the tip guard from the Tip Holder before installing the dispense cassette.

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. The 1536 Cassette's cover plate is made of steel, instead of Ultem® (polyetherimide) like the other cassette types. Remove the temporary **Tip Guard** before installation.
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. On the MicroFlo it slides into grooves on the right side 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.

Prerequisites:

- Review the **Tubing Cassette Diagram** above to learn the names of the components.
 - Move the **Pump Cover** away from the pump to its **OFF** position.
 - Release the pump's stainless steel plate. It will hang loosely from the pump.
1. Slide the **Center Holder** into its slot on the right side of the pump. A tab on the back plate of the center holder fits into a notch.




Figure 5: Slide the Center Holder into place.

2. Slide the **Tip Holder** into the dispense arm. Do not twist the tubing. The Tip Holder's front plate with the BioTek logo faces the Center Holder. Make sure the tip holder is level and snapped into place.



Figure 6: Slide the Tip Holder into the dispense arm.

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 and click them into place.

 When installing the cassettes, guide the steel plate first to the right to align with the tensioner, then, push both pieces clockwise around the pump.

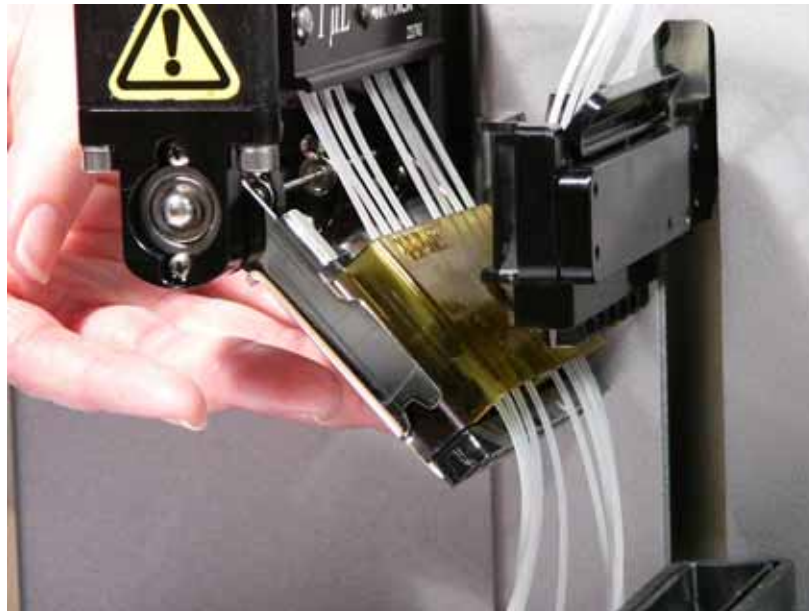


Figure 7: Align the Tube Tensioner with the steel plate.



Figure 8: Guide the Tube Tensioner and steel plate into position.

4. Lift the **Tube Organizer** over the pump cover. Place it in the fluid vessel, when you're ready.

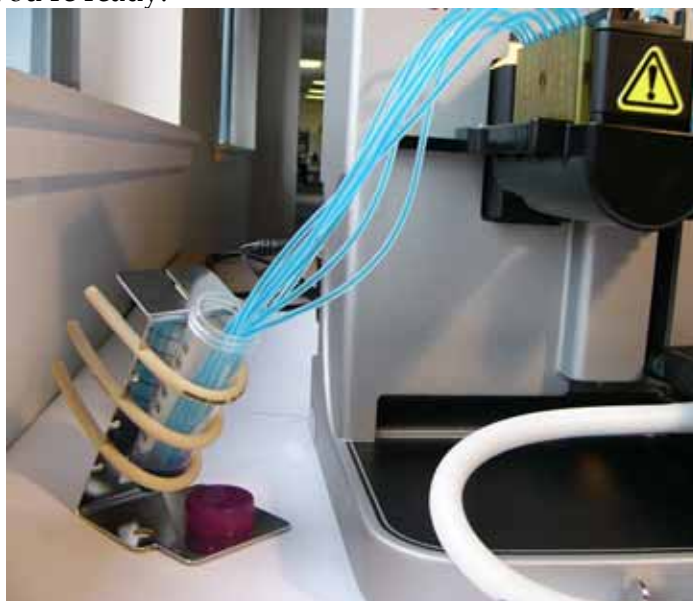


Figure 9: Put the Tube Organizer in the fluid supply vessel.

5. Return the **Pump Cover** to its **RUN** position covering the pump.

Insert the Prime Trough Drain Tube

The MicroFlo ships with a tube to drain the priming trough. You must supply a vessel to catch the fluid.

1. Plug one end of the drain tube into the spout on the priming trough.
2. Snake the tube through the bracket on the front of the dispenser and slide the plate carrier to its extreme left and right positions to make sure the tube does not impede its movement or catch an edge of the instrument. Remove any slack, if necessary.

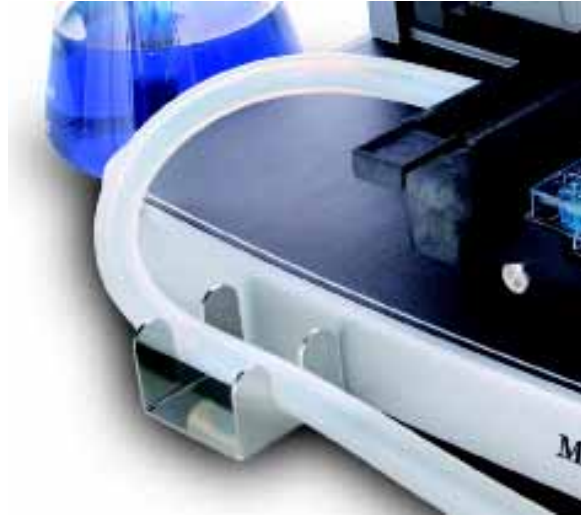


Figure 10: Prime Trough Drain Tube

Alternative Option: You can change the position of the bracket that holds the drain tube. The bracket can be secured to the back left corner of the dispenser to redirect the waste tube if that position better accommodates your workspace. You will find two screw holes in the bottom of the instrument to hold the bracket.

3. Put the other end of the tube into your waste vessel.

Prime Trough Insert

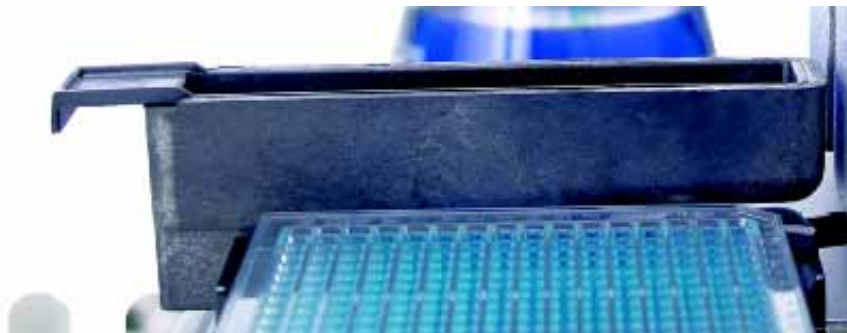


Figure 11: Prime Trough Insert for capturing precious fluids.

The MicroFlo ships with an extra reservoir to capture expensive reagent after priming, rather than discarding it. The Prime Trough Insert (PN 7172062) fits inside the prime trough.

Power Supply and Cords

The MicroFlo Select dispenser uses an external 24-volt power supply that is compatible with 100 - 250 V~; 50-60 Hz. Connect the power cord to the power supply, then connect the power supply's output plug to the 24-VDC connector on the left side of the instrument. Plug the 3-prong end of the power cord into an appropriate power receptacle.



Warning! Power Rating. The MicroFlo power supply 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.

Data Communication

Communication with Computer or Other Devices

You will find a 9-pin serial (RS-232) port and a USB port located on the left side of the instrument. These communication ports (COM Ports) allow the dispenser to communicate with the:

- BioStack™ Microplate Stacker
- Liquid Handling Control™ (LHC) software
- Robotics software application.

❖ The MicroFlo ships with a USB cable (PN 75108) to communicate with a computer for downloading basecode or using the LHC. The USB cable cannot be used to control the BioStack. The BioStack Alignment Kit includes the required serial cable for this purpose.

Operating the MicroFlo with the BioStack

Alignment hardware and serial cable connections need to be installed to operate the MicroFlo Select with the BioStack™ Microplate Stacker. Refer to your ***BioStack Operator's Manual*** for installation instructions.

4: Verify Performance

Before using the MicroFlo Select for the first time, verify that it is operating properly by turning it on. The power switch is located on the dispenser's left side.

Each time the dispenser is turned on, it automatically performs a system self-test. This test checks the carrier, cassette status, pump positioning and operation. If the test passes, the Main Menu appears and the dispenser is ready for use.

If the self-test fails, the dispenser will “chirp” and display an error code. Press the **Stop** button on the keypad and then look up the code in **Appendix A, Error Codes**.

❖ **Note:** You can also run this test from the dispenser's keypad, by selecting → → **Dispenser Action Menu > UTIL > SLFCHK**.
When using the **LHC**, select **Tools > Instrument Utilities**.

For information on performance verification and IQ/OQ/PQ procedures, see **Chapter 6, Instrument Qualification**.

Repacking and shipping the MicroFlo

If you need to ship the MicroFlo Select Microplate Dispenser to BioTek for service or repair, be sure to 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 Customer Service for replacement materials.



If the MicroFlo Microplate Dispenser has been exposed to potentially hazardous material, **decontaminate** it to minimize the risk to all who come in contact with the Dispenser during shipping, handling and servicing.

Decontamination prior to shipping is required by the U.S. Department of Transportation regulations.

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.

Before shipping the instrument:

- **Decontaminate** the dispenser and its accessories as necessary (find instructions in Chapter 5).
- Obtain a **Return Materials Authorization (RMA)** number from BioTek TAC. Mark this number on the outside of the shipping box.
- Include a **description** of what is required of the BioTek Service Department. Clearly state whether the unit requires calibration, cleaning, periodic maintenance, warranty work, and/or repair.
- Provide BioTek with the **name and telephone number** of a person who may be contacted if questions arise.
- **Insure** the instrument for full value.

Repackaging the instrument and its accessories:

Refer to the illustrations on page 25 when repackaging the MicroFlo.

- Remove the tubing cassette.
- Disconnect the prime trough drain tube.
- Install the shipping hardware (as described below).
- Use a rubber band to secure the Pump Cover in place.

Illustration 1

- Place the foam pad into the inner box.
- Place the dispenser into the foam bag and then into the inner box.
- Slide the box insert in behind the instrument.

Illustration 2

- Place the upper shipping insert into the inner box.
- Securely tape the inner box closed.

Illustration 3

- Place the eight foam corner blocks on the inner box.
- Place the inner box (with corner blocks) into the outer shipping box.
- Securely tape the shipping box closed.
- Record the RMA on the side of the shipping box.

Install the Shipping Hardware

After removing the cassette and the drain tube, you can install the shipping brackets.

Two shipping brackets and a rubber band protect the MicroFlo during shipping. Installation instructions advise attaching the shipping brackets to the back of the dispenser. They should be screwed to the left back corner of the MicroFlo. Contact BioTek Customer Care if you need replacement brackets.

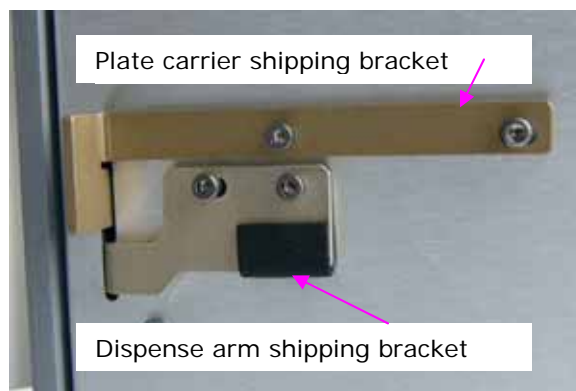


Figure 12: Shipping hardware stored on rear panel.

Required Materials

- Hex wrench (which should be stored in the small plastic storage pouch attached to the back of the instrument)

Affix the shipping brackets

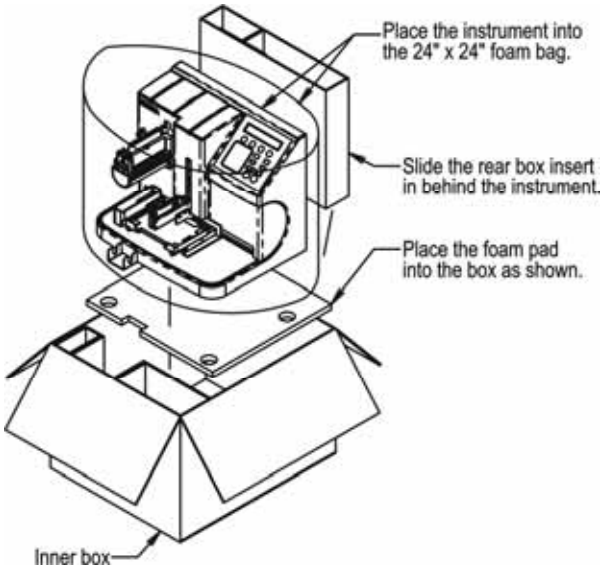
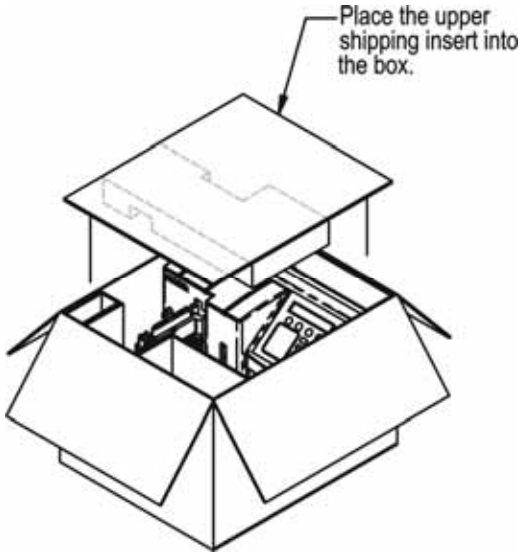
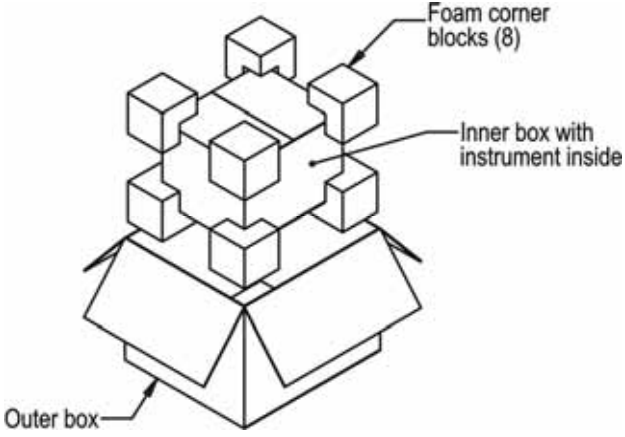
1. Turn off the MicroFlo.
2. To install the plate carrier shipping bracket, slide the plate carrier to the left. Line up the U in the bracket's short branch with the back rail and use the hex wrench to screw the long branch to the plate carrier's rail. (See the picture on page 12).



Removing the plate carrier may make it easier to install the plate carrier shipping bracket. Restore the carrier before installing the dispense arm bracket.

3. To install the dispense arm shipping bracket, put the hole in the bracket's short arm over the back rail so that the black foam patch on the wider branch protects the plate carrier from being scratched. Lower the dispense arm to align the screw holes and use the hex wrench to secure it. The bracket will hold the dispense arm just above the plate carrier.
4. Close the Pump Cover and secure it in place with the rubber band.

Repacking Illustration

| | |
|---|--|
|  <p>Place the instrument into the 24" x 24" foam bag.</p> <p>Slide the rear box insert in behind the instrument.</p> <p>Place the foam pad into the box as shown.</p> <p>Inner box</p> |  <p>Place the upper shipping insert into the box.</p> |
|  <p>Foam corner blocks (8)</p> <p>Inner box with instrument inside</p> <p>Outer box</p> | <ul style="list-style-type: none"> • Securely tape the boxes closed. • Write the RMA in large, clear letters on the outside of the outer box and return to: <p>BioTek Instruments, Inc. ATTN: RMA# xxxxx 100 Tigan Street Highland Park Winooski, VT 05404-0998 USA</p> |

Chapter 3

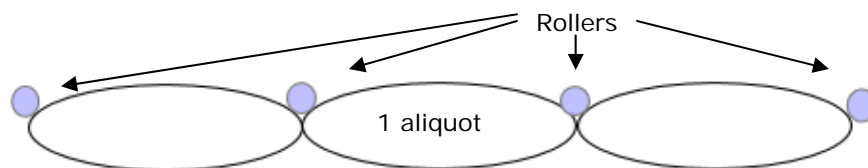
Essential Concepts

This chapter expands on the information about the MicroFlo Select Microplate Dispenser provided in the Introduction. It describes hardware and software features.

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How it works

The MicroFlo Select dispenses fluid using a peristaltic pump. It works by expelling a portion of fluid trapped between advancing rollers. The shape of the tubing between the rollers is similar to sausage links in a continuous strand.



The volume of fluid in a single sausage 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 sausages). The volume of each aliquot 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, 1 μL dispenses when using the 1 μL cassette, 5 μL dispenses when using the 5 μL cassette, and 10 μL dispenses when using the 10 μL cassette.

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 MicroFlo's 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.

Review the **Specifications** on page 7 to learn the expected life of the tubing cassettes.

How to optimize performance

Prime before dispensing. Priming the tubing is the most critical factor in assuring optimal performance. You must balance the requirement to prime the tubing with the desire to preserve expensive reagents.

The **best habit** to develop to keep the MicroFlo performing at its best and to preserve fluids is **Purging** the fluid at the end of a dispense run and **Priming** to remove large air bubbles from the tubing before dispensing. The tubing is permeable to air. When 20 minutes or more has elapsed between dispenses, or less than 20 minutes when using 1 μ L cassettes, it is important to thoroughly prime the tubing before dispensing.

In addition to purging, you can use the **priming trough insert** to capture expensive reagents for reuse.

Developing good practices like performing all the dispenses required for a certain fluid in a single session to minimize downtime and the need to prime is recommended.

Other best practices:

- **Filter the dispense fluid** to 50 microns before dispensing with the 1 μ L 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 μ L for the 1 μ L cassette, 5 μ L for the 5 μ L cassette, and 10 μ L for 10 μ L 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 μ L tubing. The best practice is to unload the 1 μ L cassette when dispensing is completed.
- **Minimize prime volume** or preserve expensive reagents by observing the tubing during a Prime and stopping its flow as soon as all the large air bubbles are removed. Pre-dispense primes can use minimum volumes or be skipped when priming is done immediately before the dispense.
- To more quickly dispense to **384-** and **1536-**well plates **change the dispense Pattern to Row.**

1536-deep-well plates

For users of 1536-well microplates, especially those designed for imaging, with wells recessed deep within the microplate: you can better position the tips inside the plate, close to the wells' rims by removing the two outer shields from the Tip Holder and lowering the Dispense Height.

How to remove the Tip Holder shields:

- 1 Look for the break-away notches etched into the two remaining shields on the outer edge of the Tip Holder.
- 2 You can use your thumb and forefinger to snap the tab off and away from the tips, but the job is easier when a small set of pliers, a wrench, or similar tool is used to get a good grip on the tab.

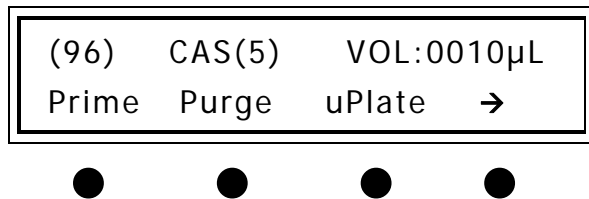


You can remove the outer shields if necessary.

Quick Dispense or Protocol?

When using its keypad, the MicroFlo gives you two ways to create, run, and save dispense programs: **Quick Dispenses** and **Protocols**. Each one has advantages and disadvantages depending on your working environment and dispense requirements.

Quick Dispense (Main Menu)



The MicroFlo stores up to 10 **Quick Dispense** programs for quick retrieval and execution. The dispenser ships with one quick program, which is displayed in the Main Menu at startup.

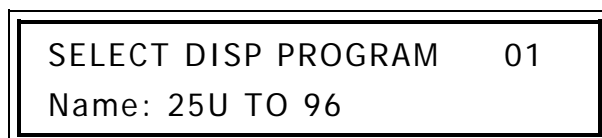
Whenever you change a dispense parameter and run the routine, the program is saved as a **Quick Dispense** program. Up to 10 distinct programs are stored. New programs displace the oldest ones. The Quick Dispense programs do not have a name, which distinguishes them from the **Protocols**. And, depending on how many you create, they may not be permanently saved.

The last used program is always ready-to-run. It is shown in the top line of the Main Menu with the plate type, cassette type, and dispense volume, as in the example shown above. The **Options** button scrolls through the Quick Dispense programs beginning with the most recently run.

The easy access and instant nature of the Quick Dispenses are obvious advantages. They are perfect for one-time-only dispense routines. And, labs with simple dispensing requirements and only one or two users may find they perfectly meet their needs.

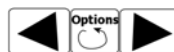
However, **Quick Dispenses** do not reveal all the parameters, which can be deceptive. A coworker may have used the MicroFlo with the same top level parameters: plate type, cassette, and volume, but changed the plate map. In labs with multiple users, running **Protocols** may be more efficient.

Protocols



Top line: Program #

Second line: Program Name



Use **Options** button to scroll

In contrast to the **Quick Dispense** programs, the MicroFlo permanently saves **Protocols**. Up to 99 Protocols can be created and stored onboard.

Protocols obey the common rules for creating and saving data: create a program, give it a unique name, define its parameters, and save it. The program is then ready to run, and can also be edited, copied, or deleted.

Another difference between Quick Dispenses and Protocols is the range of settings open to definition. A Protocol lets you select the plate type, plate map (columns-to-dispense-to), dispense volume, flow rate, dispense height, horizontal dispense position, and priming parameters. Defining a Quick Dispense is limited to volume, plate type, and plate map. The default values are applied for the other parameters.

| Available Settings | Protocols | Quick Dispense |
|---------------------|-----------|----------------|
| Dispense Volume | √ | √ |
| Plate Type | √ | √ |
| Plate Map | √ | √ |
| Flow Rate | √ | |
| Dispense Height | √ | |
| Plate Clear Height | √ | |
| Horizontal Position | √ | |
| Prime Volume | √ | |
| Prime Cycles | √ | |

Protocols offer some of the convenience of a Quick Dispense. Prior to running a selected protocol, the MicroFlo displays its dispense volume and plate map. You can temporarily change one of these values. Upon completion of the run, the MicroFlo displays a “**Repeat**” option to let you instantly run it again on another plate (with the temporarily changed values, if applicable).

BioTek recommends using **Protocols** for specific applications, regularly run assays, and to comply with GLP. Especially when the MicroFlo is being used by multiple people, protocols will give you more confidence than a **Quick Dispense** that the desired dispense parameters have been preserved.

Learn more about Quick Dispense on page 42, and more about creating and running protocols on page 43.

Handling Special Plates and Test tube racks

The MicroFlo supports numerous 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 MicroFlo's accessible design makes this task easy. You'll enjoy the flexibility it offers, especially for cell-based assays.

The MicroFlo'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, define 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, i.e., the volume you input, will be half the desired volume. As always, be sure to also consider the MicroFlo's optimal dispense volumes 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 Horizontal 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: two tubes can easily fit in these large wells, so all 8 tubes can be used, 2 per well.
- **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.

Test Tube Racks

The MicroFlo supports two types of test tubes that fit in a test tube rack (PN 7170520) available from BioTek as an accessory item:


- 20 - 12 mm X 75 mm, 6 mL capacity tubes. Select **75T** when using the keypad.
- 20 - 13 mm X 100 mm, 9 mL capacity tubes. Select **100T** when using the keypad.

Before dispensing to a test tube rack, the odd-numbered dispense tubes must be removed from either the fluid supply or from the cassette itself. Only the even-numbered channels are properly positioned to dispense to the tubes.

❖ **Remove channels 1, 3, 5, and 7 from the cassette or fluid supply before dispensing to test tubes.**

You must also remove the plate carrier and replace it with the test tube rack. Follow the instructions to **Install the Test Tube Rack** in **Appendix B**.

| Plate Type | Columns/Rows | Tubes per Well | Tubes removed |
|----------------|--------------|----------------|---------------|
| 20 75mm Tubes | 5 x 4 | 1 | 1, 3, 5, 7 |
| 20 100mm Tubes | 5 x 4 | 1 | 1, 3, 5, 7 |

 If you regularly use the special plates or test tubes that require some of the 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 on the operator's manual CD titled: **7171017_(current Rev)_Replacing the tubing_8x14.PDF** to learn how to remove the tubes.

Chemical Compatibility

For your own safety and to ensure the dispenser's long life, follow these guidelines when choosing compounds for use in the MicroFlo.

❖ **Note:** We advise against continuous contact with harsh chemicals. It is best if the dispenser is rinsed with deionized water after contact with any strong acid, base, or solvent.

The following materials are used in the MicroFlo Select dispenser and tubing cassettes. Materials listed as 1-10 in **Table 1** are cross-referenced as headings 1-10 in **Table 2**.

Table 1. Material/Where Used List

| # | Material | Where Used |
|----|--------------------------------|--|
| 1 | 316 Stainless Steel | Steel dispense tips, Tip Holder cover plate on 1536 cassette, pump rollers, carrier rails, cassette bolts. |
| 2 | Aluminum | Center Holder, Tube Tensioner, pump head, dispense arm |
| 3 | Brass | Threaded inserts in Center Holder and Tip Holder |
| 4 | Polycarbonate | Plate carrier, pump shroud |
| 5 | Polypropylene | Molded dispense tips |
| 6 | Polystyrene | Assay plates |
| 7 | PTFE (polytetrafluoroethylene) | Plate carrier |
| 8 | Ryton® PPS | Tube Organizer, priming trough, priming trough insert |
| 9 | Silicone | Tubing |
| 10 | Ultem (polyetherimide) | Center Holder, Tube Tensioner, Tip Holder |

Table 2. Chemical Compatibility Ratings

| Key | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |
|--------------------------|--------------|--|-------|-------------|-------------|-------------|------|-------|------------------|-------|
| | 316 S. Steel | Aluminum | Brass | Polycarbon. | Polypropyl. | Polystyrene | Ptfe | Ryton | Silicone | Ultem |
| Chemical | | (1-10 represent Materials listed in Table 1 above) | | | | | | | | |
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |
| Acetic Acid, 5% | A | B | D | A | A | D | A | A | A | A |
| Acetic Anhydride | A | A | D | D | B | D | A | A | A | ND |
| Acetonitrile | A | B | ND | D | A | D | A | A | D ⁽¹⁾ | D |
| Ammonia 10% | A | A | ND | D | A | B | A | A | D | D |
| Benzyl Alcohol | B | B | ND | ND | A | D | A | A | A | ND |
| Chloroform | A | B | B | D | C | D | A | A | D | D |
| Detergents 1% | A | B | ND | A | A | A | A | A | A | A |
| Dimethylformamide | B | A | ND | D | A | D | A | A | A | ND |
| DMSO (Dimethylsulfoxide) | A | A | ND | D | A | D | A | A | C ⁽¹⁾ | D |
| Ethyl Alcohol 70% | A | A | A | B | A | A | A | A | B | A |
| Ethylene Oxide | B | D | D | C | D | C | A | D | A | ND |
| Formaldehyde 37% | A | B | A | A | A | ND | A | A | C | A |
| Hexane | A | A | A | D | B | D | A | A | D | A |
| Hydrochloric Acid 20% | D | D | D | B | B | C | A | D | D | A |
| Hydrofluoric Acid 20% | D | D | ND | D | A | ND | A | A | D | ND |
| Hydrogen Peroxide 10% | B | A | D | A | A | A | A | C | A | A |
| Isopropyl Alcohol 70% | A | A | A | A | A | A | A | A | A | A |
| Methyl Alcohol 70% | A | A | A | B | A | ND | A | A | A | A |
| Methylene Chloride | B | C | A | D | B | D | A | A | D | D |
| Phosphoric Acid 10% | D | C | D | A | A | B | A | A | C | A |
| Propylene Glycol | B | B | ND | B | A | A | A | A | A | ND |
| Sodium Chlorate | B | C | ND | A | A | ND | A | A | C | ND |
| Sodium Hydroxide 20% | B | D | B | A | A | A | A | A | A | A |
| Sodium Hypochlorite <20% | C | D | D | C | A | A | A | C | B | B |
| Sodium Hypochlorite 0.5% | B | D | D | C | A | A | A | C | B | A |
| Sulfuric Acid <10% | B | B | ND | A | A | A | A | A | C | A |
| Trichloroethylene | B | D | ND | ND | C | D | A | A | D | D |
| Virkon 10% | A | D | ND | A | A | A | ND | A | A | ND |

- ❖ (1) Exposure to DMSO and Acetonitrile may cause the silicone tubing to swell, increasing the volume of fluid dispensed. The magnitude of this effect will vary with concentration and exposure. Re-calibration of the cassette may be required.
- ❖ Please contact BioTek with questions about compatibility of any chemicals not described here.

Chapter 4

Operation

This chapter provides instructions for operating the MicroFlo Select Microplate Dispenser using the keypad. When using the Liquid Handling Control™ (LHC) software refer to its Help system to learn how to control the MicroFlo.

| | |
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Basic Operation

This section introduces you to the MicroFlo's controls and options for running dispense programs.

❖ You may want to begin by reading the discussion of the two methods for defining, saving and running dispenses: **Quick Dispense** and **Protocols** in the *Essential Concepts* chapter.

Turn On/Off the MicroFlo

To turn the MicroFlo on or off, press the On/Off switch on the dispenser's left side:

| = On ○ = Off

The dispenser performs a self-test. If the test passes, the Main Menu will appear and the dispenser is ready for use. If the self-test fails, the dispenser will "chirp" and display an error code. Press the **Stop** key. Look up the code in **Appendix A: Error Codes**.

Using the Keypad

The MicroFlo features a 25-key keypad and a 2-line x 24-character LCD display as the user interface. The keypad is shown below.

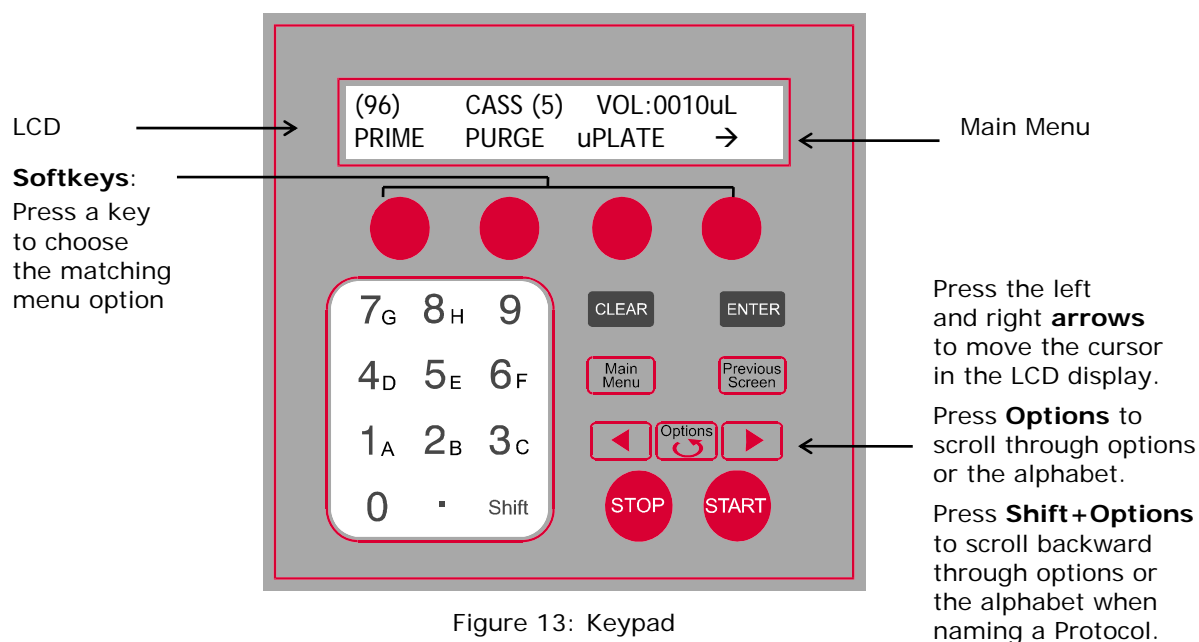
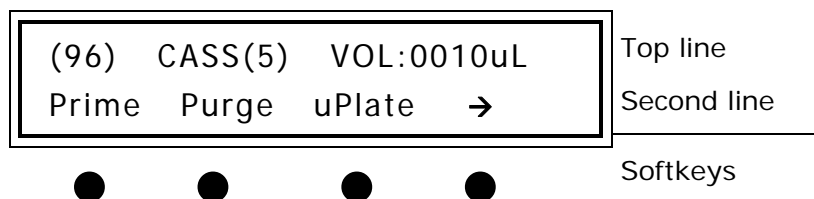


Figure 13: Keypad

Main Menu

The MicroFlo's main menu is displayed at startup.



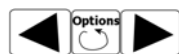
The two line display shows a **Quick Dispense** in the top line and menu options in the second line.

BioTek ships the dispenser from the factory with one Quick Dispense program (described below) installed. It is displayed in the top line:

- (96) is the plate type
- CASS (5) is the cassette type
- VOL: shows the dispense volume per well




When all the three values: plate type, cassette type, and volume are accurate, put a plate on the carrier and press **Start** to run the dispense.



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.

To select a menu option in the second line of the MicroFlo's display, press the **SoftKey** on the keypad below it:

- **Prime**: press and hold down the Softkey to prime the tubing. Fluid flows into the prime trough for as long as you press the key.
- **Purge**: press and hold down the Softkey to purge the tubing. Fluid is pumped back into the supply vessel as long as you press the key.
- **uPlate**: press the Softkey to change plate type and/or plate map, i.e. the columns of the plate to dispense to. Learn more on page 51.
- **→** : press the Softkey under the arrow to change menus. The MicroFlo has three menus: **Main Menu**, **Dispenser Run Menu**, **Dispenser Action Menu**.
-  : Press the **Main Menu** button one time to display the current menu; When necessary, press the **Main Menu** button twice to return to the main menu.

Stop Program

Press the **STOP** key to stop running (abort) a program.

Move Backward Through the Menus

To move to a previous menu, press the **Previous Screen** key.

Scroll Through Options

Certain functions, such as entering a program name or selecting a program, offer a set of options from which to make a selection. To view the different options, press the **Options** key or the **Shift + Options** key combination. Press the **ENTER** key to select the current option.

Dispenser Run Menu

When using the MicroFlo's keypad, from the main menu press the arrow key once for this menu:



Press its **SoftKey** to select a menu option displayed in the second line of the display:

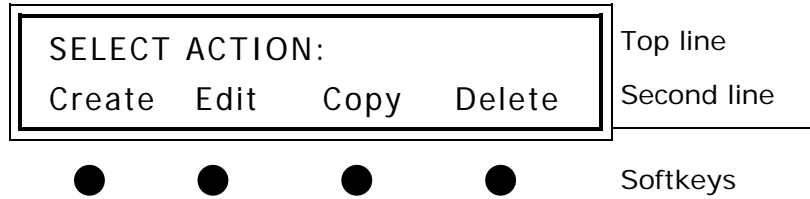
- **Run:** select this option to run one of the **Protocols**. Enter the protocol's number in the top line, or use the **Options** button to scroll through the list of programs. Press **Enter** to select it and follow the prompts. The plate map and dispense volume can be changed for the current run. Upon completion of the run you can put another plate on the carrier and select **Repeat**. Or, select **New** to change the parameters or select another program.
- **Prime:** press and hold down the Softkey to prime the tubing. Fluid flows into the prime trough for as long as you press the key.
- **Purge:** press and hold down the Softkey to purge the tubing. Fluid is pumped back into the supply vessel as long as you press the key.
- **→ :** press the Softkey under the arrow to move to the next menu: **Dispenser Action Menu**.

Dispenser Action Menu

From the main menu press the arrow key one time for this menu:



Define



Use the **Define** menu to create and edit Protocols. You can also use **Copy** to create a new program. Select **Delete** to remove unneeded programs.

Copy a Protocol

1. Select **Copy** from the Define menu,
2. Enter the program's number in the top line, or use the Options button to scroll through the list of programs. Press Enter to select it.
3. Enter a new/unique name for the program. The MicroFlo will assign it the next available number.
4. Back at the **Define** menu, select **Edit** to modify the new program.

Util

The Utility option offers System Self Check and CheckSum: see **Utility Menu Options** on page 54.

Setup

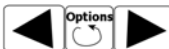
The Setup menu holds configuration and other critical details: see Setup Menu Options on page 53.

Run a Quick Dispense

| | | | |
|-------|----------|------------------|---|
| (384) | CASS(10) | VOL:0010 μ L | |
| Prime | Purge | uPlate | → |



To run a saved Quick Dispense:

1. Put the microplate on the carrier.
2.  Use the **Options** button to scroll through and select a quick program. (Up to 10 distinct programs are stored.)
3. When the desired program is displayed, press **Start**.

To create a Quick Dispense:

[Change the volume](#), plate type or map, and click **Start**. The program will be saved as the newest Quick Dispense.

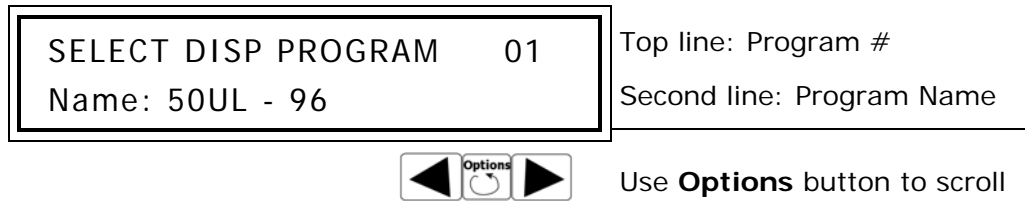
About Quick Dispense Programs

The MicroFlo stores up to 10 Quick Dispense programs for quick retrieval and execution. The last used program is always ready-to-run. It is shown in the top line of the Main Menu with the plate type, cassette type, and dispense volume, as in the example shown above.

Quick Dispenses perform a two-cycle pre-prime immediately before execution. The prime volume matches the cassette volume and two primes are performed. A Quick Dispense using a 5 μ L cassette will be preceded by two pre-dispense primes of 5 μ L, for example.

Whenever you define/change dispense parameters and run the routine, the program is saved for reuse. New programs displace the oldest ones. These programs are not named, which distinguishes them from the **Protocols**. Read a comparison of the Quick Dispense and Protocols in the Essential Concepts chapter.

About Dispense Protocols



In contrast to the **Quick Dispense** programs selected at the keypad's main menu, the MicroFlo's **Protocols** require a unique name, using up to 16 alphanumeric characters. You create **Protocols** by defining and saving the dispense parameters. Up to 99 Protocols can be stored onboard the instrument.

When using the LHC, an unlimited number of Protocols can be saved on your computer.

- ❖ See a comparison of the Quick Dispenses and Protocols in the *Essential Concepts* chapter.

To create or modify a Protocol using the keypad:

- ❖ Use the Liquid Handling Control™ (LHC) software's Help system to learn how to create or modify protocols using the LHC.

1. At the main menu select → (by pressing the arrow's Softkey) twice to get to the **Dispenser Action Menu**.
2. Select **Define**.
3. Choose an option: **Create** or **Edit**. (Learn about the **Copy** and **Delete** on page 41.)
4. When creating a program, enter a **Name**: (See below). When editing a program use the **Options** key to scroll to the program's number. Press **Enter** to select the program or save the name.
5. Starting with the plate type, define each parameter and press **Enter** to save your input and move to the next option. Use the table below to learn more about each parameter.
6. Use the Softkeys to select **Yes** or **No** to the last prompt: OK to save program?
Return to the Dispenser Run Menu (press **Previous Screen**) to run the program.

Dispense Protocol Parameters

| Dispense Parameters | |
|-------------------------------|--|
| Name and Number | <p>Using the keypad: When creating a protocol onboard using the keypad, the MicroFlo assigns the first available number to newly created protocols. You can assign a descriptive name, but protocols are selected for editing and running by their number. Learn more, including how to enter a name on page 45.</p> <p>Using the LHC: When creating a protocol using the LHC, enter a unique name in the Protocol Name field. And, while they can be different, BioTek recommends using the same name when saving the protocol file. The file will be saved with a .LHC filename extension.</p> |
| Plate | <p>Using the keypad: Follow the µPlate instructions on page 51.</p> <p>Using the LHC: refer to the LHC's Help for instructions.</p> |
| Dispense Volume | Enter the amount of fluid in microliters (µL) to dispense to each well. (See also: Special Plate Handling, 12-, 48-well plates and test tubes in the <i>Essential Concepts</i> chapter.) |
| Flow Rate | <p>Low, Medium, or High:</p> <p>Low rate is recommended when using PCR or round-bottom plates to avoid fluid splashing.</p> <p>High rate is recommended for faster throughput.</p> |
| Dispense Height | The distance between the bottom of the well and the dispense tips when dispensing. |
| Plate Clearance Height | <p>The height the dispense arm moves to after dispensing to a column and while it moves to the next column.</p> <p>The height is set in steps from 0 to 2048. Refer to the manufacturer's specifications when setting the height for non-standard vessels. Always keep the tips at least 2 mm above the surface.</p> <p>Use the two height settings to position the tips inside the wells when dispensing, and raise the tips safely above the plate during transitions. Angled dispensing and lowering the tips into the wells may minimize disturbance to cell layers in applicable assays.</p> <p>Using the keypad: Press Clear to restore default values for the current plate type. To adjust the height, enter the approximate number of steps, assess the value in millimeters, and adjust accordingly.</p> <p>Using the LHC: Click Show advanced options and select the button to "Use the default" height or choose a custom</p> |

| Dispense Parameters | |
|-----------------------------|---|
| | height by entering the number of steps or millimeters. |
| Horizontal Position | <p>From a central axis of 0, you can adjust the plate position to dispense to one side of the well. This may be useful for some cell-based assays. Some trial and error is most likely needed to find the optimal position.</p> <p>Increase the number of steps to move the plate to the left, so the dispense tips address the right side of the wells. Decrease the number of steps, using a negative number, to move the plate carrier to the right, so the dispense tips address the left side of the wells.</p> <p>For all plate types, the default value is 0 (zero) for the horizontal offset.</p> <p>Using the keypad: With the number of steps set to 0, hold the Shift key and press Options to enter a negative number.</p> <p>Using the LHC: Click Show advanced options and use the text field to enter the number of steps, or use the scroll keys. The corresponding distance in millimeters is displayed.</p> |
| Prime Before Start? | Select Yes to prime the tubing before dispensing to the plate, then define the prime values. Otherwise, select No to not perform a prime before dispensing. |
| Prime Volume | Enter the amount of fluid in microliters (μL) to pump through each tube into the priming trough per prime cycle. The recommended prime volume is 10 times the cassette size: 10 μL for 1 μL cassette; 50 μL for 5 μL ; 100 μL for 10 μL . |
| Number Pre-Dispenses | Enter the number of prime cycles to perform before beginning the dispense. |

❖ **Important:** Priming the dispenser after it has been idle for 20 or more minutes, especially when using the 1 μL cassette, is essential for achieving accurate dispense volumes. Press the **Prime** button on the dispenser or add a Prime to an LHC protocol.

Protocol Names

Onboard the MicroFlo, protocols are tracked by a number because the keypad does not offer a way to easily search for a protocol by its name. (The opposite is true when using the LHC computer control.) Up to 99 Protocols can be saved onboard the dispenser. (And, unlimited number can be saved on your PC.)


The MicroFlo assigns the first available number to a newly created protocol. The dispenser ships without any pre-defined protocols, so the first one you create will be numbered 01. The second will be numbered 02, and so on.

When using the keypad, protocols are selected for a run and for editing by their number, so the MicroFlo retains the number it assigns. When a program is deleted, the remaining programs are **not** renumbered. The deleted program's position is filled with the next created program or left blank.

This numbering convention holds true when transferring protocols from the computer to the instrument using the LHC's **Transfer Protocols** feature.

How to name a protocol using the keypad:

| | |
|------------------|------------------------------|
| NAME: _ | Top line: Enter Name |
| - % & _ | Second line: Select a symbol |



Use **Options** button to scroll through alphabet

- Press **Shift** + key A-H, or scroll through the alphabet with the **Options** key for A-Z. **Shift + Options** reverses direction.
- ◀ ▶ Use the arrow keys to move the cursor within the display. It underlines the current space.
- The second line of the **NAME** display offers four symbols - % & _ that can be used in a protocol name: dash (hyphen), percent sign, ampersand, and underscore. Press its corresponding SoftKey to include the symbol in the name.

Press **ENTER** to store the completed name and continue. A protocol name must be unique. If the name already exists, the dispenser will require you to change it. This protects programs from inadvertently being overwritten.

Clear Entry

To delete an entry, press the **CLEAR** key.

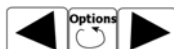
Run a Dispense Protocol

→ Dispenser Run Menu > RUN

| | |
|---------------------|----|
| SELECT DISP PROGRAM | 01 |
| Name: 50UL – 96 | |

Top line: Program #

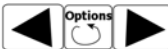
Second line: Program Name



Use **Options** button to scroll

❖ When using the Liquid Handling Control (LHC) software, put the plate on the carrier before pressing **Run**. Learn more in the LHC's Help system.

To run a Protocol using the keypad:

1. At the main menu select → (by pressing the arrow's Softkey) to get to the **Dispenser Run Menu**.
2. Select **Run**.
3.  Use the **Options** button to scroll through and select a protocol or enter a protocol number. (Up to 99 can be stored onboard.) Be sure to select a protocol that is optimal for your cassette type.
4. Press **Enter** to select the protocol.
5. You can change the plate map, if desired. Press **Enter** to save the map and move to the next option.
6. You can change the dispense volume, if desired. Press **Enter** to save the volume and proceed.
7. Follow the prompt: put the microplate on the carrier and press **Start** to run the protocol.

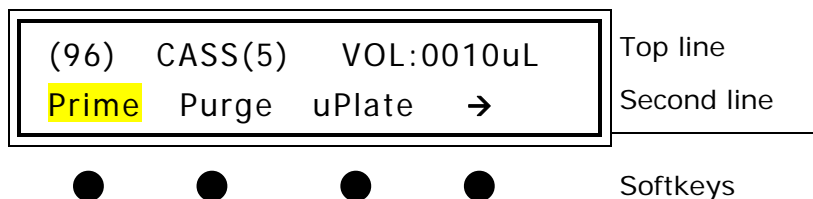
❖ **Important:** Priming the dispenser after it has been idle for 20 or more minutes, especially when using the 1 µL cassette, is essential for achieving accurate dispense volumes. Press the **Prime** button on the dispenser in between runs (or add a Prime step to an LHC protocol). Learn 'How to optimize performance' in the *Essential Concepts* chapter.

Prime the MicroFlo

Prime the MicroFlo to remove air bubbles from the tubing prior to dispensing fluid, especially when the dispenser has been idle for more than 20 minutes between runs.

Anytime Prime

Use the Softkey at MicroFlo's main menu to prime the dispenser anytime:



Press and hold the **Prime** key to flush the tubing. Fluid flows into the prime trough for as long as you press the key.

❖ For top efficiency, Purge the fluid at the end of run, and Prime the tubing before the run.

Pre-Prime

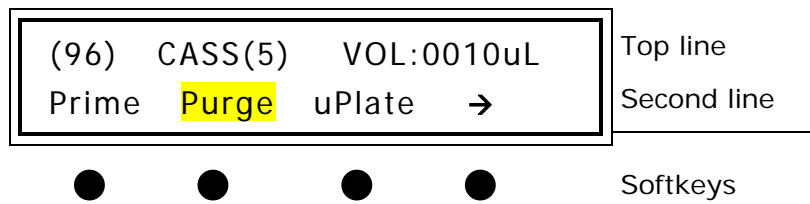
Pre-priming before a dispense run to condition the tips is a standard operating procedure. MicroFlo's **Quick Dispense** programs include 2 pre-prime cycles before each run. You cannot change the prime settings of the Quick Dispenses. But, MicroFlo's **Protocols** let you define the pre-priming volume and number of cycles.

Purge the MicroFlo

Purging reclaims the fluid in the tubing, reversing its direction, and moving it back to the supply vessel. In addition to using the Purge to preserve expensive reagent, it's an effective tool for eliminating air bubbles in the tubing. Alternating between purging and priming is a good exercise for removing air bubbles.

Purge

Use the Softkey at MicroFlo's main menu to purge the dispenser:



Press and hold the **Purge** key to reverse the direction of the fluid and return it to the supply vessel. Press and hold the key until all the fluid in the tubing is evacuated.

Using the LHC

You can use BioTek's Liquid Handling Control™ (LHC) software to purge the MicroFlo before or after a dispense run by adding a Purge Step to the protocol. Consult the LHC's Help to learn more.

Removing air bubbles from the tubing:

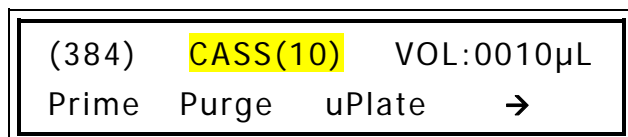
When the dispenser has been idle for 20 minutes or more:

1. Purge the tubing to move fluid in reverse direction.
2. Prime the tubing to flush fluid through the tubing.
3. Repeat this pattern until all air bubbles are removed from the tubing.

Define the Cassette

→ → Dispenser Action Menu > SETUP > CASS

It is important to correctly identify the cassette type for accurate dispensing. The current cassette setting is shown in the main menu.



CASS(#) in the top line of the LED displays the current Cassette setting. Likewise, the cassette is prominently labeled. Make sure the MicroFlo's setting matches the currently used cassette.

The MicroFlo displays a warning message when an out-of-range volume is defined for the current cassette. For example, if you request a 150 µL dispense with a 1 µL cassette, a message will require you to confirm your intention or cancel the run.

To define the cassette type:

1. At the MicroFlo's main menu, select → twice to get to the **Dispenser Action Menu**.
2. Select **SETUP**.
3. Select **CASS**.
4. Select the **1 µL**, **5 µL**, or **10 µL** cassette.

This action returns you to the Setup Options. The cassette type is changed to your selection. Confirm this at the main menu: press **Main Menu** twice.

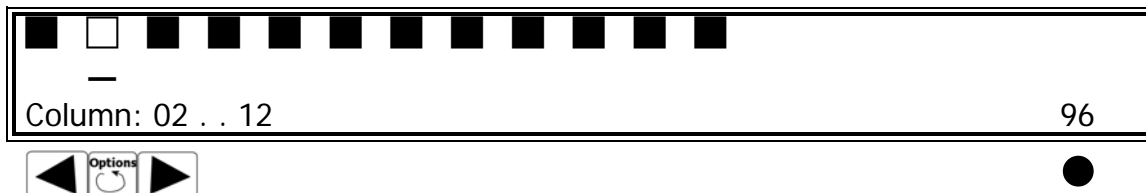
Removing the Tubing Cassette

❖ Review the Tubing Cassette Diagram on page 14.

1. Lift the **Tube Organizer** out of the fluid vessel.
2. Move the **Pump Cover** away from the pump to its off position.
3. Release the stainless steel plate holding the Tube Tensioner: release the catch on the top front of the pump that holds it in place. It will hang loosely.
4. Lift the **Center Holder** out of its slot on the right side of the pump.
5. Hold the Center Holder in one hand while you slide the **Tip Holder** out of the dispense arm.

Define the Plate Type and Plate Map


1. At the main menu select **uPlate** with the Softkey.



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. The current plate type is shown in the right corner of the second line. The left corner of the second line shows the range of columns, beginning with the currently selected column.

To change the plate type:

2. Repeatedly press the Softkey under the plate type (in the far right corner of the display) to scroll through the available options. Hold the Shift key while pressing the button to reverse direction.

 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.

To change the plate map:

3. ◀ ▶ Use the arrow keys to move the cursor to the column you want to define. The cursor underlines the currently selected column and its number is shown in the display. In the example above, column 2 is currently selected.
4. Press the **Options** key to toggle between filling the column or not. When the image of the column in the display is filled it will be dispensed to. Conversely, when the column image is blank or unfilled, the column will not be dispensed to.
5. Press **Main Menu** to save the settings and run a dispense.

About Plate Types and Plate Maps

The MicroFlo stores the details required to process more than a dozen **Plate Types**, including a test tube rack (optional accessory). The optimal dispense height and horizontal position are defined onboard for each plate type. The MicroFlo permits adjustments to these values when necessary for special situations. For general use, the predefined parameters allow instant dispensing to all plate types.

The MicroFlo's **Plate Map** defines which column or range of columns to dispense to. The graphic representation of the columns makes it easy to pick and choose the plate's columns. Plate Maps can be changed on-the-fly for both **Quick Dispenses** and **Protocols**.

Plate Types Table

| Plate Type | Plate Name | Columns x Rows | Dispense Height | | Tubes per Well |
|-----------------|------------|----------------|-----------------|-------|----------------|
| | | | Steps | mm | |
| 96 Well | 96 | 12x8 | 336 | 15.36 | 1 |
| 384 Well | 384 | 24x16 | 336 | 15.36 | 1 |
| 1536 Well | 1536 | 48x32 | 254 | 11.61 | 1 |
| 384 PCR | 384P | 24x16 | 254 | 11.61 | 1 |
| 96 Mini Tubes | 96MT | 12x8 | 1124 | 51.40 | 1 |
| 96 Deep Well | 96D | 12x8 | 964 | 44.07 | 1 |
| 384 Deep Well | 384D | 24x16 | 964 | 44.07 | 1 |
| 20 12x75 Tubes | 75T | 5x4 | 1433 | 65.52 | 1* |
| 20 13x100 Tubes | 100T | 5x4 | 1989 | 90.94 | 1* |
| 6 Well | 6 | 3x2 | 488 | 22.31 | 4 |
| 12 Well | 12 | 4x3 | 488 | 22.31 | 2* |
| 24 Well | 24 | 6x4 | 488 | 22.31 | 2 |
| 48 Well | 48 | 8x6 | 488 | 22.31 | 1* |

*** Important:** when dispensing to test tubes, and 6-, 12- and 48-well plates some dispense tubes must be removed from the fluid supply vessel. See **Special Plates Handling** on page 33.

Fill Pattern

When using **384-** and **1536-well** plates, the MicroFlo offers two dispense patterns: Column or Row. For these 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, if applicable. Row-wise processing is faster because it requires fewer changes in direction. Once it is defined, your **Pattern** preference will apply to all runs, Quick Dispenses and Protocols.

Using the keypad: Set the fill pattern using the **Dispenser Action Menu>Setup**.

Using the LHC: Select **Tools>Instrument Utilities** to set the fill pattern

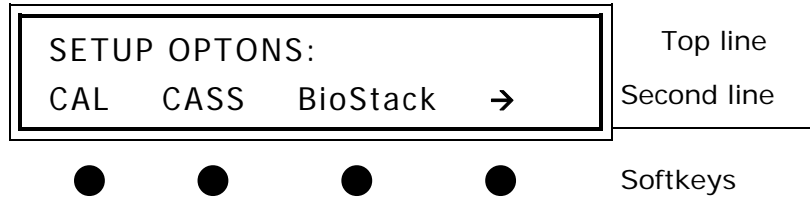
Test Tube Racks

BioTek provides a test tube rack for use with the MicroFlo as optional accessory. It supports dispensing to two common test tube sizes. Contact your BioTek representative to purchase one.

Be sure to follow instructions for removing the plate carrier and properly replacing it with the test tube rack in **Appendix B**.

Setup Menu Options

→ → Dispenser Action Menu > SETUP



The Setup menu holds configuration and other critical details:

- **CAL:** short for calibrate, is an aid to calibrating the MicroFlo on our factory floor. It is not needed when calibrating cassettes gravimetrically or when using the Cassette Calibration Kit.
- **CASS:** short for cassette, tells the MicroFlo which cassette type is installed. This is critically important for expected performance. The size of the tubing determines the amount of fluid dispensed per well.
- **BioStack:** offers controls for using the BioStack to process plates with the MicroFlo. See *Using the BioStack* on page 55.
- **Pattern:** is only applicable when processing 384- and 1536-well plates. It lets you select the fill pattern: **Column** or **Row**. Select Row for faster throughput.

Utility Menu Options

→ → Dispenser Action Menu > UTIL

Self-Test

Each time it is turned on, the MicroFlo automatically runs a self-check to verify system components. It can also be run from the Utility menu.

The self-check verifies movement of the dispense arm and plate carrier.

Startup: When the test completes successfully at startup the Main Menu appears. If the test fails, the dispenser will “chirp” and display an error code. Jot down the **error code** and turn off the dispenser. Fix the problem and turn it on again.

Utility Menu: When the test completes successfully from the Utility menu a “passed” message is displayed. If the test fails, the dispenser will “chirp” and display an error code. Press the **Enter** key, look up the **Error Code** and fix the problem. Re-run the SLFCHK.

❖ Error Codes are listed in **Appendix A**.

Checksum

Recording the Checksum is a regular part of the instrument qualification procedures and useful when troubleshooting. The MicroFlo has two internal processors, the User Interface (**UI**) to control the LED display and communicate with a computer (when applicable) and the Motor Controller (**MC**)

1. At the MicroFlo’s main menu, select → twice to get to the **Dispenser Action Menu**.
2. Select **UTIL**.
3. Select **CHKSUM**.

| | | |
|------------------------|----|----------------|
| Version Checksum Info: | | |
| UI | MC | STKR(BioStack) |



4. Select **UI**, record the code displayed and press **Enter** to return to the Checksum options. Repeat for **MC**. When running a BioStack, select **STKR** to obtain the BioStack’s version information.

Serial Number

Select **SN** to view the instrument’s serial number.

Using the BioStack

→ → Dispenser Action Menu > BIOSTACK

If you have purchased BioTek's BioStack™ Microplate Stacker to operate with the MicroFlo, follow the instructions in your *BioStack Operator's Manual* to set it up to interact with the dispenser.

After installing the BioStack to operate with the MicroFlo, use the MicroFlo's menu to:

- **Configure:** tell the MicroFlo to use the BioStack.
- **Align:** align the BioStack's gripper with the MicroFlo's plate carrier.
- **Verify:** test the setup.

Set the MicroFlo to use the BioStack:

After installing the BioStack, tell the MicroFlo to use it to process plates:

1. At the main menu, press → twice to get to the **Dispenser Action Menu**
2. Select **SETUP**
3. Select **BioStack**
4. Select **Conf**



5. At the Operating Mode menu, select **BioStack**.

Repeat these steps, except select **Manual**, to run the MicroFlo without the BioStack.

Align the BioStack with the MicroFlo:

The BioStack's installation instructions include an alignment step. Use the MicroFlo's menu to access the BioStack Alignment Utility:

1. At the main menu, press → twice to get to the **Dispenser Action Menu**
2. Select **SETUP**
3. Select **BioStack**
4. Select **Align**

Follow instruction provided in the BioStack Operator's Manual.

Chapter 5

Preventive Maintenance

This chapter provides step-by-step instructions for maintaining the MicroFlo Select Microplate Dispenser in top condition, to ensure that it continues to perform to specification.

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Overview

The level of the maintenance required to keep the MicroFlo 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. Similarly, when using the 1 μ L cassette fluids should be filtered 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, as well as 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.

Recommended Maintenance Schedule

| Tasks | Page | Daily | Monthly | As needed |
|---|------|-------|---------|----------------------------|
| Flush Tubing Cassette | 59 | √ | | |
| Record The Approximate Number Of Plates Processed | 60 | √ | | |
| Clean Exterior Surfaces | 61 | | | √ |
| Clean Prime Trough And Drain Tube | 61 | | √ | |
| Verify Performance | 62 | | √ | |
| Recalibrate Cassette | 65 | | | √ |
| Unclog Dispense Tips | 63 | | | √ |
| Replace Tubing | 65 | | | √ |
| Decontaminate | 66 | | | Before shipping or storage |

Flush the Tubing Cassette

Daily Maintenance involves priming the tubing with an appropriate reagent at the beginning of the day, and, flushing 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. 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 DI water.
 - When dispensing protein solutions, first prime the tubing with a buffered saline solution to remove protein particles, then, prime with DI water.
3. Hold the **Prime** button on the keypad:
 - 1 μ L cassette = 5 seconds
 - 5 μ L cassette = 12 seconds
 - 10 μ L cassette = 6 seconds.

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 Types | Cassette Life | Total Volume |
|----------------------|---|--------------|
| 1 μL | 1000 384-well plates @ 5 μL /well | 2,000 mL |
| 1 μL 1536 | 250 1536-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 |

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 |
| 10/10/07 | 26 | 384 | 5 μL | 49920 μL | 50 mL |
| 10/11/07 | 33 | 96 | 10 μL | 31680 μL | 82 mL |
| | | | | | |

Clean Exterior Surfaces

Exposed surfaces may be cleaned (not decontaminated) with a cloth moistened (not soaked) with water or water and a mild detergent. You'll need:

- Deionized or distilled water
- Clean, lint-free cotton cloths
- Mild detergent (optional)

To clean the exposed surfaces:

1. **Important!** Turn off and unplug the instrument.
2. Moisten a clean cotton cloth with water, or with water and mild detergent. **Do not soak the cloth.**
3. Wipe the plate carrier and all exposed surfaces of the instrument.
4. If detergent was used, wipe all surfaces with a cloth moistened with water.
5. Use a clean, dry cloth to dry all wet surfaces.

Clean Priming Trough and Drain Hose

Remove the priming trough, priming trough insert, and the priming trough drain hose and soak them in warm water and a mild detergent. You'll need:

- Deionized or distilled water
- Clean, lint-free cotton cloths
- Mild detergent

To clean these accessories:

1. **Important!** Turn off and unplug the instrument.
2. Remove the drain tube from the priming trough and waste container.
3. Slide the prime trough towards you and away from the dispenser.
4. Soak the trough, trough insert and drain tube in hot sudsy water for at least a half hour.
5. Rinse them thoroughly, and wipe the exterior surfaces with a clean, dry cloth.

Verify Performance

It is important to regularly verify dispense performance. A good test of the MicroFlo's performance occurs in the lead up to calibrating the cassette. Perform the initial steps of one of these calibration procedures to verify performance. The results will determine the need to actually recalibrate the cassette:


- Use the **Cassette Calibration Kit** described in the PDF on the Operator's Manual CD titled: **7171009_(current Rev)_Calibration Kit Instructions.PDF**.
- Use the **Gravimetric Calibration** method described in Appendix C of the Operator's Manual.

Unclogging 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 μ L 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).

Replace the 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 in the PDFs folder on the MicroFlo Operator's Manual CD 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 Cassette

Cassette Calibration Kit

BioTek offers an accessory to the MicroFlo, a Calibration Kit that speeds up the process of recalibrating a cassette. It is also useful to verify performance, one of the recommended monthly maintenance routines.

Follow the instructions shipped with the kit or find them in on the Operator's Manual CD 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 in **Appendix C**.

Decontamination

Purpose

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.



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 Biohazard(s) they handle.



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 an alternative to bleach)
- Deionized or distilled water
- Safety glasses
- Clean cotton cloths
- Surgical mask
- Protective gloves
- Lab coat
- Biohazard trash bags

Decontamination Procedure



Warning! Internal Voltage. Always turn off and unplug the instrument for all decontamination and cleaning operations.



The bleach solution is caustic; wear gloves and eye protection when handling this solution.

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 Service Department.

Do not soak the keypad. This will cause damage. Wipe the keypad with a damp cloth.

1. Turn off and unplug the MicroFlo Select.
2. Unload the cassette and the prime trough drain tube.
3. Autoclave the cassette.
4. Prepare an aqueous solution of 0.5% sodium hypochlorite (bleach). As an alternative, 70% isopropyl alcohol may be used if the effects of bleach are a concern.

❖ **Note:** Be sure to check the % NaClO of the bleach you are using; this information is printed on the side of the bottle. Commercial bleach is typically 10% NaClO; if this is the case, use a 1:20 dilution. Household bleach is typically 5% NaClO; if this is the case use a 1:10 dilution.

❖ **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.**
6. Wipe the plate carrier, priming trough, dispense arm, and the other exposed surfaces, except the keypad.
7. Wait 20 minutes. Moisten a cloth with DI or distilled water and wipe all surfaces of the instrument that have been cleaned with the bleach solution or alcohol.
8. Use a clean, dry cloth to dry all wet surfaces.
9. Soak the drain tube in hot soapy water or the remaining bleach solution for twenty minutes or more. Rinse well and set aside to dry.
10. Discard the used gloves and cloths using a Biohazard trash bag and an approved Biohazard container.

Chapter 6

Instrument Qualification

Testing is conducted on every MicroFlo Select Microplate Dispenser to verify dispense accuracy and precision before it leaves BioTek.

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IQ/OQ/PQ

Installation Qualification (IQ) confirms that the dispenser and its components have been supplied as ordered and ensures that they are assembled and configured properly for your lab environment.

- The recommended IQ procedure consists of setting up the instrument as described in **Chapter 2, Installation** and then performing the System Self-Check and the Checksum Test.
- The IQ procedure should be performed *initially* (before the dispenser is used for the first time).
- The successful completion of the IQ procedure verifies that the instrument is installed correctly. The Operational Qualification procedure (see below) should be performed immediately following the successful IQ.

Operational Qualification (OQ) confirms that the dispenser operates according to specification initially and over time.

- The recommended OQ procedure consists of performing the System Self-Check, the Checksum Test, and the appropriate Dispense Precision and Accuracy Tests.
- Your facility's operating policies may also require that you perform an actual assay prior to accepting the dispenser for routine use. You should not use the data obtained from the first assay that utilizes the dispenser until you have confirmed that the package insert criteria have been met.
- The OQ procedure should be performed before first use and then annually. It should also be performed after any major repair or upgrade to the hardware or software.
- Although out-of-tolerance failures will be detected by the OQ tests, results should be compared with those from the monthly Performance Qualification tests and previous OQ tests to monitor for trends.
- The successful completion of the OQ procedure, in combination with results that are comparable to previous PQ and OQ tests, confirms that the dispenser is performing consistently over time.

Performance Qualification (PQ) confirms that the dispenser consistently meets the requirements of the tests performed at your laboratory.

- The recommended PQ procedure consists of performing the System Self-Check, the Checksum Test, and the Dispense Precision and Accuracy Test.
- Your facility's operating policies may also require that you routinely perform an actual assay, to confirm that the dispenser will consistently give adequate results for the assays to be run with it.
- These tests should be performed routinely; the recommended interval is *monthly*. This frequency may be adjusted depending on the trends observed over time.
- The successful completion of the PQ procedure confirms that the dispenser is performing consistently under normal operating conditions.

Recommended Qualification Schedule

| Task/Test | IQ | OQ | PQ |
|--------------------------------------|-----------|----------------------|---------|
| | Initially | Initially & Annually | Monthly |
| Installation and Setup | ✓ | | |
| System Self-Check | ✓ | ✓ | ✓ |
| Checksum Test | ✓ | ✓ | ✓ |
| Dispense Precision and Accuracy Test | | ✓ | ✓ |
| Run Assay | | ✓ | ✓ |

System Self-Check and Checksum Test

System Self-Check

Each time it is turned on, the MicroFlo Select automatically runs the System Self-Check to verify system components. The self-check verifies movement of the dispense arm and plate carrier.

Using the Keypad

Select → → **UTIL> SLFCHK**

Using the LHC

Select **Tools> Instrument Utilities> Self Check**

When the test completes successfully a “passed” message is displayed. If the test fails, the dispenser will “chirp” and display an error code.

Checksum Test

Using the Keypad

Select → → **UTIL> CHKSUM**

Select **UI**, record the version code displayed and press **Enter** to return to the Checksum options. Repeat for **MC**.

Using the LHC

Select **Tools> Instrument Utilities> Version** (click the Version link to refresh the checksum information).

Record the version and checksum information.

Dispense Precision and Accuracy Tests

Overview

Dispense precision is a measure of the variability of volumes dispensed from tube to tube across the manifold. The optical density of the solution in a well is proportional to the total volume of the solution in the well. If 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.

The accuracy specifications for the cassettes are intended to describe the performance of tubing throughout its life. Typically, achieving a dispense accuracy that is 1/10th of the specified accuracy is the goal of calibration.

Dispense accuracy is computed gravimetrically using this formula:

$$Accuracy (\%) = 100 \frac{(m_{actual} - m_{expected})}{m_{expected}}$$

Dispense Precision and Accuracy

| Cassette | Precision | Accuracy |
|----------|---------------------------|-----------------------|
| 1µL | < 10%CV @ 1µL per well | ± 10% @ 1µL per well |
| | < 5%CV @ 2µL per well* | ± 5% @ 2µL per well* |
| 1µL 1536 | < 10%CV @ 1µL per well | ± 10% @ 1µL per well |
| | < 5%CV @ 2µL per well* | ± 5% @ 2µL per well* |
| 5µL | < 5%CV @ 5µL per well | ± 4% @ 5µL per well |
| | < 2.5%CV @ 10µL per well* | ± 2% @ 10µL per well* |
| 10µL | < 4%CV @ 10µL per well | ± 4% @ 10µL per well |
| | < 2%CV @ 20µL per well* | ± 2% @ 20µL per well* |

- ❖ * These specifications are for these dispense volumes and higher.
- ❖ Note: The above specifications are for performance. For IQ/PQ/OQ purposes, we have added 1.0% additional tolerance to the %CV to accommodate various test solutions, off-peak wavelengths, reader errors, and pipette errors.

Liquid Testing Methodology

The tests are fundamentally the same for all cassettes except the 1536: Tare an empty plate on a balance. Use the MicroFlo Select 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).

1 μL 1536 Cassettes: To test the 1536 cassette type, dispense the 1536 Test Solution to every other column of a 1536-well plate. Examine the wells to determine if the cassette is dispensing precisely to the selected columns with no splatter into non-selected columns or onto the plate surface. Then, perform the tests defined for the **1 μL cassette** type using a 96-well plate.

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 | | | √ | |
| 10 μL | √ | √ | | √ |
| 50 μL | | | √ | |
| 100 μL | | | | √ |
| 1536 μL | | √ | | |

- **1 μL Test:** The 1 μL test is used to confirm 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 DI 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.

- **5 μL Test:** The 5 μL test is used to confirm 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 DI water to raise the fluid level for optimal reading.
- **10 μL Test:** The 10 μL test is used to confirm 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 150 μL of DI water to raise the fluid level for optimal reading.

- **50 μL Test:** The 50 μL test is used to confirm the performance of the 5 μL cassettes when dispensing 10 aliquots (2 1/2 turns of pump) into each well of the plate. Dispense 50 μL into each well using the **50 μL solution**, and requires an additional 100 μL of DI water to raise the fluid level for optimal reading.
- **100 μL Test:** The 100 μL test is used to confirm the performance of the 10 μL cassettes when dispensing 10 aliquots (2 1/2 turns of pump) into each well of the plate. Dispense 100 μL into each well using the solution called **100 μL solution**, and requires an additional 50 μL of DI water to raise the fluid level for optimal reading.
- **1536 Test:** This test is intended to check the alignment of the fluid jets produced by the 1536 well cassettes. Given the small diameter wells of the 1536 well plates, it is important to confirm that the tips in the cassette are firing straight into the wells. Dispense 6 μL into every other column of a 1536 well plate using the solution called **1536 solution**. Then, perform the **1 μL Test** described above.

Materials

- Corning Costar #3590 96-well plates, or equivalent
- 1536-well plates (when testing 1536 models), 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 405 (or 450) nm

❖ If you are using one of BioTek's keypad-based absorbance readers, such as the ELx800™ or ELx808™, ensure that the reader is not running in Rapid mode. To check this, select UTIL > READ and cycle through the prompts until you see READ IN RAPID MODE?"

- BioTek blue dye solution, PN 7773001, or equivalent to create the test solutions described on the following page. See also the important notes below:

❖ BioTek determined the pass/fail specifications for the following tests using the test solutions described. You may choose to use your own solutions. If any tests fail using your own solutions, however, retry the tests using the suggested solutions.

❖ 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 1.300 OD.
- ❖ Using pure DI water in place of these solutions is **not** recommended and will likely result in the failure of the unit to meet specifications.
- ❖ Liquid Test Worksheet templates can be found at the end of this chapter for recording data reduction results. If your tests are failing, this information will be useful for BioTek TAC to help diagnose any problems.

Test Solutions

Unique concentrations of the test fluid are described here, each one corresponds to a specific dispense volume, except the 1536 Solution for 1536-well testing. Prepare the solutions you will need to validate the cassette types and dispense volumes used most commonly in your applications.

- ❖ The 5 μ L Solution is used to make the higher volume test solutions.

1 μ L Solution

Using BioTek's 10X concentrated blue dye solution (PN 7773001), mix 5 mL of DI H₂O with 8 mL of the blue dye solution.

5 μ L Solution

Using BioTek's 10X concentrated blue dye solution (PN 7773001), mix 100 mL of DI H₂O with 10 mL of the blue dye solution.

10 μ L Solution

Mix 25 mL of DI H₂O with 20 mL of the **5 μ L Solution** (described above).

50 μ L Solution

Mix 45 mL of DI H₂O with 5 mL of the **5 μ L Solution** (described above).

100 μ L Solution

Mix 40 mL of DI H₂O with 2 mL of the **5 μ L Solution** (described above).

1536 Solution

Mix 5 mL of 70% Isopropyl Alcohol with 3 mL of the **5 μ L Solution** (described above) and 35 mL of DI H₂O.

Performing the Precision and Accuracy Tests

Prerequisite:

- Prepare the test solutions as described on page 76.
- Make a copy of the applicable worksheet beginning on page 80.

Procedure:

1. Install the cassette to be tested.
2. Turn on the MicroFlo Select and make sure the cassette 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:
 - 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
 - Retain the default settings for Dispense Height, Plate Clear Height and Horizontal Position.
 - Define a **Prime before start**: set the volume to 10 μL .
 - Set the **Number of Pre-dispenses** to 2.
 - **1536 Test** only: : Change the **plate map** to dispense to every other column. It will be easier to observe the dispense results than dispensing to the whole plate.
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, add the specified amount of de-ionized water to each well to raise the fluid level to approximately 150 μL , except for the 1536 Test:

| 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.

❖ If you are using one of BioTek's keypad-based absorbance readers, such as the ELx800™ or ELx808™, ensure that the reader is **not** running in Rapid mode. To check this, select UTIL > READ and cycle through the prompts.

12. Calculate the **Delta OD**: (630nm - 450nm), **Mean Absorbance**, **Standard Deviation**, and the **%CV** for the wells under test. **%CV** = (Standard Deviation \div 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 the instructions for Unclogging the Tips in the **Maintenance** chapter). 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

The following pages contain Dispense Precision & Accuracy Test Worksheets. We recommend you make copies of the appropriate pages and use them to record your calculations and test results.

Alternatively, you can purchase the **MicroFlo Select Qualification & Maintenance Procedures Kit** from BioTek, which contains additional tools for conducting test procedures and recording the results, including logbooks and Microsoft® Excel® spreadsheets.

MicroFlo Select Dispense Precision & Accuracy Test Worksheet 1 μ L Cassette Test

| 1 μ L Dispense Precision Test | |
|---|---|
| Standard Deviation (<i>SD</i>): | |
| Mean Absorbance (<i>sum of all wells \div 96</i>) | |
| % CV (<i>SD \div Mean \times 100</i>) | % |
| % CV must be < 11.0% | <input type="checkbox"/> Pass <input type="checkbox"/> Fail |

| 1 μ L Dispense Accuracy Test | |
|---|---|
| Total Dispense (Actual) Weight: | grams |
| Expected Weight: (<i>mL/well \times number of wells dispensed</i>) | grams |
| % Accuracy Error: (<i>Actual Weight – Expected Weight</i>) \div <i>Expected Weight \times 100</i> | % |
| % Accuracy Error must be < 10.0% | <input type="checkbox"/> Pass <input type="checkbox"/> Fail |
| Visual verification that no well varies considerably from the others | <input type="checkbox"/> Pass <input type="checkbox"/> Fail |

| | |
|--------------------------------|--|
| Cassette Serial Number: | |
| Tests Performed By: | |
| Date: | |
| Reviewed/Approved By: | |
| Date: | |

MicroFlo Select Dispense Precision & Accuracy Test Worksheet 5 µL Cassette Test

| 5 µL Dispense Precision Test | |
|--|---|
| Standard Deviation (<i>SD</i>): | |
| Mean Absorbance (<i>sum of all wells ÷ 96</i>) | |
| % CV (<i>SD ÷ Mean x 100</i>) | % |
| % CV must be < 6.0% | <input type="checkbox"/> Pass <input type="checkbox"/> Fail |

| 5 µL Dispense Accuracy Test | |
|---|---|
| Total Dispense (Actual) Weight: | grams |
| Expected Weight: <i>(mL/well x number of wells dispensed)</i> | grams |
| % Accuracy Error: <i>(Actual Weight – Expected Weight) ÷ Expected Weight x 100</i> | % |
| % Accuracy Error must be < 4.0% | <input type="checkbox"/> Pass <input type="checkbox"/> Fail |
| Visual verification that no well varies considerably from the others | <input type="checkbox"/> Pass <input type="checkbox"/> Fail |

| | |
|--------------------------------|--|
| Cassette Serial Number: | |
| Tests Performed By: | |
| Date: | |
| Reviewed/Approved By: | |
| Date: | |

MicroFlo Select Dispense Precision & Accuracy Test Worksheet 1 μ L Cassette Test

| 10 μ L Dispense Precision Test | |
|---|---|
| Standard Deviation (<i>SD</i>): | |
| Mean Absorbance (<i>sum of all wells \div 96</i>) | |
| % CV (<i>SD \div Mean \times 100</i>) | % |
| % CV must be < 6.0% | <input type="checkbox"/> Pass <input type="checkbox"/> Fail |

| 10 μ L Dispense Accuracy Test | |
|---|---|
| Total Dispense (Actual) Weight: | grams |
| Expected Weight: (<i>mL/well \times number of wells dispensed</i>) | grams |
| % Accuracy Error: (<i>Actual Weight – Expected Weight</i>) \div <i>Expected Weight \times 100</i> | % |
| % Accuracy Error must be < 5.0% | <input type="checkbox"/> Pass <input type="checkbox"/> Fail |
| Visual verification that no well varies considerably from the others | <input type="checkbox"/> Pass <input type="checkbox"/> Fail |

| | |
|--------------------------------|--|
| Cassette Serial Number: | |
| Tests Performed By: | |
| Date: | |
| Reviewed/Approved By: | |
| Date: | |

MicroFlo Select Dispense Precision & Accuracy Test Worksheet 10 µL Cassette Test

| 10 µL Dispense Precision Test | |
|--|---|
| Standard Deviation (<i>SD</i>): | |
| Mean Absorbance (<i>sum of all wells ÷ 96</i>) | |
| % CV (<i>SD ÷ Mean x 100</i>) | % |
| % CV must be < 5.0% | <input type="checkbox"/> Pass <input type="checkbox"/> Fail |

| 10 µL Dispense Accuracy Test | |
|---|---|
| Total Dispense (Actual) Weight: | grams |
| Expected Weight: <i>(mL/well x number of wells dispensed)</i> | grams |
| % Accuracy Error: <i>(Actual Weight – Expected Weight) ÷ Expected Weight x 100</i> | % |
| % Accuracy Error must be < 4.0% | <input type="checkbox"/> Pass <input type="checkbox"/> Fail |
| Visual verification that no well varies considerably from the others | <input type="checkbox"/> Pass <input type="checkbox"/> Fail |

| | |
|--------------------------------|--|
| Cassette Serial Number: | |
| Tests Performed By: | |
| Date: | |
| Reviewed/Approved By: | |
| Date: | |

MicroFlo Select Dispense Precision & Accuracy Test Worksheet 5 μ L Cassette Test

| 50 μ L Dispense Precision Test | |
|---|---|
| Standard Deviation (<i>SD</i>): | |
| Mean Absorbance (<i>sum of all wells \div 96</i>) | |
| % CV (<i>SD \div Mean \times 100</i>) | % |
| % CV must be < 3.5% | <input type="checkbox"/> Pass <input type="checkbox"/> Fail |

| 50 μ L Dispense Accuracy Test | |
|--|---|
| Total Dispense (Actual) Weight: | grams |
| Expected Weight: (<i>mL/well \times number of wells dispensed</i>) | grams |
| % Accuracy Error: (<i>Actual Weight – Expected Weight</i>) \div <i>Expected Weight</i> \times 100 | % |
| % Accuracy Error must be < 2.0% | <input type="checkbox"/> Pass <input type="checkbox"/> Fail |
| Visual verification that no well varies considerably from the others | <input type="checkbox"/> Pass <input type="checkbox"/> Fail |

| | |
|--------------------------------|--|
| Cassette Serial Number: | |
| Tests Performed By: | |
| Date: | |
| Reviewed/Approved By: | |
| Date: | |

MicroFlo Select Dispense Precision & Accuracy Test Worksheet 10 µL Cassette Test

| 100 µL Dispense Precision Test | |
|--|---|
| Standard Deviation (<i>SD</i>): | |
| Mean Absorbance (<i>sum of all wells ÷ 96</i>) | |
| % CV (<i>SD ÷ Mean x 100</i>) | % |
| % CV must be < 3.0% | <input type="checkbox"/> Pass <input type="checkbox"/> Fail |

| 100 µL Dispense Accuracy Test | |
|---|---|
| Total Dispense (Actual) Weight: | grams |
| Expected Weight: <i>(mL/well x number of wells dispensed)</i> | grams |
| % Accuracy Error: <i>(Actual Weight – Expected Weight) ÷ Expected Weight x 100</i> | % |
| % Accuracy Error must be < 2.0% | <input type="checkbox"/> Pass <input type="checkbox"/> Fail |
| Visual verification that no well varies considerably from the others | <input type="checkbox"/> Pass <input type="checkbox"/> Fail |

| | |
|--------------------------------|--|
| Cassette Serial Number: | |
| Tests Performed By: | |
| Date: | |
| Reviewed/Approved By: | |
| Date: | |

MicroFlo Select Dispense Precision & Accuracy Test Worksheet 1536 Cassette Test

| 1536 Dispense Precision Test | |
|--|---|
| Standard Deviation (<i>SD</i>): | |
| Mean Absorbance (<i>sum of all wells ÷ 96</i>) | |
| % CV (<i>SD ÷ Mean x 100</i>) | % |
| % CV must be < 6.0% | <input type="checkbox"/> Pass <input type="checkbox"/> Fail |

| 1536 Dispense Accuracy Test | |
|--|---|
| Total Dispense (Actual) Weight: | grams |
| Expected Weight: (<i>mL/well x number of wells dispensed</i>) | grams |
| % Accuracy Error: (<i>Actual Weight – Expected Weight</i>) ÷ <i>Expected Weight</i> x 100 | % |
| % Accuracy Error must be < 5.0% | <input type="checkbox"/> Pass <input type="checkbox"/> Fail |
| Visual verification that no well varies considerably from the others | <input type="checkbox"/> Pass <input type="checkbox"/> Fail |

| | |
|--------------------------------|--|
| Cassette Serial Number: | |
| Tests Performed By: | |
| Date: | |
| Reviewed/Approved By: | |
| Date: | |

Chapter 7

Troubleshooting

This chapter offers suggestions for troubleshooting performance problems with the MicroFlo Select Microplate Dispenser.

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| Performance Issues..... | 88 |
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| Contact BioTek Technical Assistance Center..... | 90 |

Performance Issues

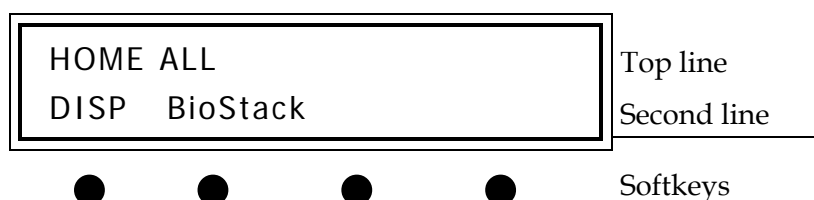
| Issue | Solution |
|----------------------------------|---|
| Fluid stream missing wells | <p>Check Tip Holder, make sure it is properly seated in the Dispense Arm.</p> <p>Select the correct Plate Type.</p> <p>Tips are clogged. (See Unclogging Tips in the Preventive Maintenance chapter.)</p> |
| Fluid splashing out of the wells | <p>Select the correct Plate Type.</p> <p>Reduce the Flow Rate (only available when defining a Protocol, not a Quick Dispense).</p> <p>Lower the Dispense Height.</p> |
| Uneven dispensing | <p>Make sure all cassette components are properly seated in their respective positions.</p> <p>Tips are clogged. (See Unclogging Tips in the Preventive Maintenance chapter.)</p> <p>Recalibrate the cassette.</p> <p>Replace the tubing. (On the Operator's Manual CD, see 7171017_(<i>current Rev</i>)_Replacing the tubing_8x14.PDF)</p> |
| Dispenser skipping columns | <p>Check/define the Plate Map (uPlate).</p> |
| Tips clogging | <p>Filter the dispense fluid to 50 microns before dispensing.</p> <p>Replace the tubing.</p> |
| Viscous fluids sticking to tips | <p>Vary the Flow Rate: experiment with different flow rates to determine which setting best forces fluid to break from the tip.</p> <p>Clean the Tips (See the Preventive Maintenance chapter.)</p> |
| Cannot communicate with computer | <p>Check the cabling (See next page.)</p> <p>Select the correct COM Port</p> <p>Turn on dispenser; display Main Menu</p> |

Error Codes

The MicroFlo displays an error code when it detects a problem. This section describes the most common error codes and suggests action steps for resolving it. **Appendix A** provides a complete list of the error codes with a brief technical description. If this section does not cover the error code displayed by your dispenser, contact BioTek TAC (next page) for assistance.

Corrective Action

Homing the MicroFlo can reset its internal sensors and clear many errors. Jot down any error code displayed and press **Enter**. This leads to the Home All menu:



Fix the error, and:

- Press the **DISP** key to home the dispenser.
- Press the **BioStack** key to home the BioStack.

Press **Main Menu** twice when the homing is completed to return to the start screen.

Correctable Errors

| Error Code | Message | Corrective Action |
|----------------|--|---|
| 304 | Pump motor safety switch open | Close the Pump Cover |
| 401 | X-axis failed positional verify | Make sure the Prime Trough Drain Tube does not impede plate carrier movement |
| 403 | Z-axis failed positional verify | Select the proper Plate Type . Adjust the Dispense Height if using a custom plate. |
| 6040 – 6061 | Communication Error between MicroFlo and computer or LHC | See below |

Communication Errors

Take the following steps to correct any error codes between 6040-6061:

- Check USB cabling – make sure both ends are plugged in correctly
- Make sure dispenser is turned on and not busy; Main Menu is displayed
- Select the correct COM Port – learn **About COM Ports** in the LHC Help.

❖ The MicroFlo ships with a USB cable (PN 75108) to communicate with a computer for downloading basecode or using the LHC. The USB cable cannot be used to control the BioStack. The BioStack Alignment Kit includes the required serial cable for this purpose.

Contact BioTek Technical Assistance Center

If you cannot resolve technical or performance issues with the MicroFlo Select from the information provided, contact BioTek:

Service/Technical Assistance Center (TAC)

Phone: 800-242-4685 (toll free in the U.S.)
802-655-4740 (outside the U.S.)
Fax: 802-654-0638
E-Mail: tac@biotek.com

European Coordination Center/ Authorized European Representative

BioTek® Instruments GmbH
Kocherwaldstrasse 34
D-74177 Bad Friedrichshall
Germany
Internet: www.biotek.de
Phone: +49 (0) 7136 9680
Fax: +49 (0) 7136 968 111
E-Mail: info@biotek.de

Appendix A

Error Codes

This section lists the MicroFlo's error codes with a brief description of how to resolve them, if possible. Contact BioTek TAC for additional assistance.

| | |
|------------------------------|----|
| Instrument Error Codes | 92 |
| Software Error Codes | 94 |

Error Codes

This section lists two types of error codes, instrument generated errors and software errors when using the Liquid Handling Control™ (LHC) software on the computer. Instrument errors are displayed in a message box by the LHC software, or in the keypad LCD when running the dispenser manually.

- ❖ If you haven't already done so, review the **Troubleshooting** chapter for less technical descriptions of the correctable error codes and corrective action you can take to resolve them.

Instrument Error Codes

When an error code is displayed follow the prompts and try to resolve it. If the dispenser does not respond as expected, turn off the dispenser. Upon restarting the dispenser, you should be able to enter commands using the keypad or the LHC. If not, contact BioTek TAC for support.

- ❖ Use the table below to decipher the error code. For example, error code 304 means the "Pump Cover is not in the safe position (closed over the pump) for operation." This meaning is extrapolated from the error code table: 0304 <4 = Pump Motor> "interlock safety switch is open."

Motor Definitions: (shown as <motor> in error table)

- **Carrier Motor X** – 1
- **Carrier Motor Y** – 2
- **Dispense Arm Motor** – 3
- **Pump Motor** – 4

| Error Name | Code | Brief Description |
|------------------------|------|---|
| BTI_NO_ERROR | 0000 | No instrument errors detected |
| ABORT_ERROR | 0100 | Task was aborted |
| MOTOR_SENSOR_ERROR | 0200 | <motor> didn't find opto sensor transition |
| MOTOR_INTERLOCK_ERROR | 0300 | <motor> interlock safety switch open |
| MOTOR_VERIFY_ERROR | 0400 | <motor> failed positional verify |
| MOTOR_NOT_HOMED_ERROR | 0500 | <motor> not homed successfully |
| MOTOR_IN_USE_ERROR | 0600 | <motor> currently in use |
| MOTOR_NOT_FOUND_ERROR | 0700 | <motor> doesn't exist |
| CALIBRATION_ERR | 0900 | Calibration failed |
| INVALID_PLATE_TYPE_ERR | 0A00 | Invalid plate type selected |
| CNFG_DATA_ABSENT_ERR | 0C01 | Requested config/autocal data absent |
| CNFG_CHECKSUM_ERR | 0C02 | Calculated checksum didn't match saved checksum |
| CNFG_PARAMETER_ERR | 0C03 | Config parameter out of range |

| Error Name | Code | Brief Description |
|-----------------------------|------|--|
| BOOT_POWERUP_CHECKSUM_FAIL | 1001 | Bootcode powerup checksum test failed |
| BOOT_ERROR_UNKNOWN | 1002 | Unknown error in bootcode |
| BOOT_PAGE_PROGRAM_ERROR | 1003 | Bootcode page program error |
| BOOT_BLOCK_SIZE_ERR | 1004 | Bootcode block size error (not 256) |
| BOOT_INVALID_SIGNATURE | 1005 | Invalid processor signature (not 1280,1281,2560,2561) |
| BOOT_MEMORY_EXCEEDED | 1006 | Bootcode memory exceeded |
| BOOT_INVALID_SLAVE_PORT | 1007 | Invalid slave port |
| BOOT_INVALID_SLAVE_RESPONSE | 1008 | Invalid response from slave |
| BOOT_INVALID_PROCESSOR | 1009 | Invalid processor detected |
| BOOT_DOWNLOAD_CHECKSUM_ERR | 1010 | Checksum error downloading basecode |
| PARAMETER_LIMIT_ERR | 2400 | Parameter limit exceeded |
| PROGRAM_LOCKED | 4000 | Program locked so operation denied |
| PROGRAM_NONERASABLE | 4100 | Program non erasable so delete denied |
| SC_NAK_ERROR | 8100 | Communications NAK |
| SC_TIMEOUT_ERROR | 8101 | Timeout while waiting for serial message data |
| SC_COM_BUSY_ERROR | 8102 | Instrument busy and unable to process message |
| SC_RX_BUF_OVERFLOW_ERROR | 8103 | Receive buffer overflow error |
| SC_CHECKSUM_ERROR | 8104 | Checksum error |
| SC_INVALID_SCRUCT_TYPE | 8105 | Invalid structure type in byMsgStructure header field |
| SC_INVALID_DESTINATION | 8106 | Invalid destination in byMsgDestination header field |
| SC_OBJECT_NOT_SUPPORTED | 8107 | Request object received not supported by instrument |
| SC_MSG_BODY_SIZE_ERROR | 8108 | Message Body size exceeds max limit |
| SC_MAX_REQS_RUNNING_ERROR | 8109 | Max number of requests currently running and cannot run the latest request |
| SC_NO_REQS_RUNNING_ERROR | 810A | No request running when response request issued |
| SC_RESP_NOT_READY_ERROR | 810C | Response for outstanding request not ready yet |
| SC_INST_NOT_IN_PC_COMM_MODE | 810D | Instrument not in a mode to receive a PC message |
| SC_REQ_PARAMS_NOT_VALID | 810E | One or more request parameters are not valid |
| TCB_NOT_AVAIL_ERR | A000 | Task control block not available |
| DEV_NOT_AVAIL_ERR | A100 | <device> not available |
| CODE_VERSION_ERR | A200 | Version strings for multiple uProcessors do not match |
| POWER_SUPPLY_ERR | A300 | <test type> power supply level error |
| MALLOC_ERR | A400 | Malloc failed |
| DISPLAY_IN_USE | A500 | Multiple tasks attempted to use display simultaneously |

Software Error Codes

The following error codes are displayed when using the LHC software to control the MicroFlo.

| Error Code | Description |
|-----------------------------|---|
| 6000 | General communication error during download. |
| 6001 | COM port created by USB converter no longer active. |
| 6010 | The data is invalid or out-of-range. |
| 6011 | This step type can not be downloaded. |
| 6012 | Illegal characters in protocol name; valid characters are letters, numbers, spaces, or _-%& |
| 6013 | The protocol name length must be 16 characters or less. |
| 6014 | A 1536 well plate is not supported by this instrument. |
| 6015 | The specified volume exceeds the cassette maximum limit. |
| 6016 | The Volume is out-of-range. |
| 6017 | Invalid Flow rate. |
| 6018 | Invalid number of pre-dispenses. |
| 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. |
| Communication Errors | |
| 6040 | Invalid baud rate |
| 6041 | Invalid data bits selection |
| 6042 | Invalid stop bits selection |
| 6043 | Invalid parity selection |
| 6044 | Serial port error |
| 6045 | Serial write error |
| 6046 | Serial read error |
| 6047 | Checksum error |
| 6048 | Serial NAK error |
| 6049 | Excess data, or not enough data, received |
| 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 |
| 6056 | Unauthorized to open the COM port |
| 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 |

Appendix B

Dispensing to Test Tubes

This section provides detailed instructions for operating the MicroFlo Select with the optional test tube rack for dispensing to 12 mm X 75 mm, 6 mL capacity tubes or 13 mm X 100 mm, 9 mL capacity tubes.

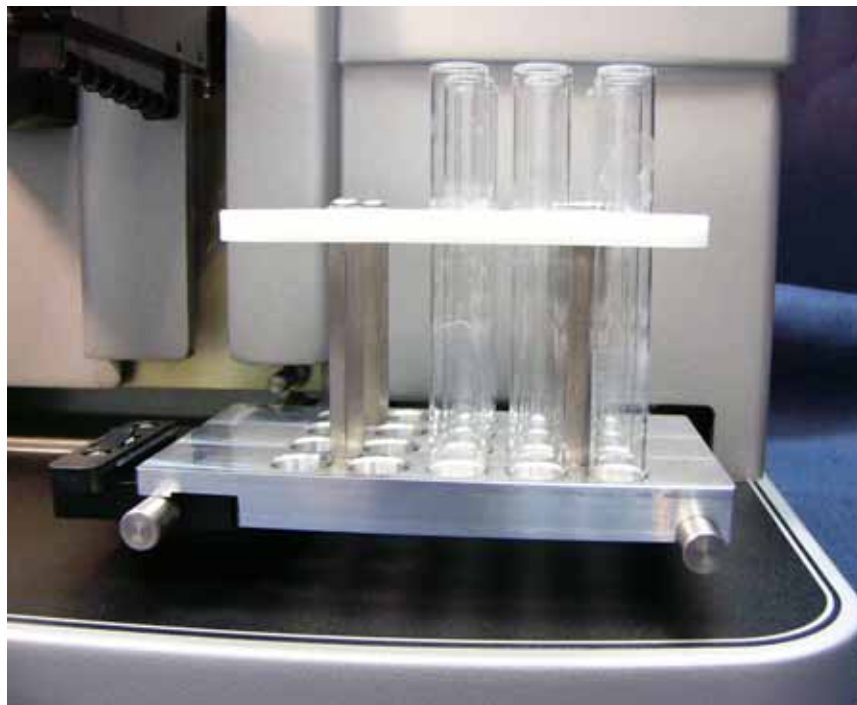
❖ **Important:** Read the Special Plates section in the *Essential Concepts* chapter before dispensing to test tubes.

| | |
|---------------------------------|----|
| Install the Test Tube Rack..... | 96 |
| Dispensing to Test Tubes | 97 |

Install the test tube rack

You must swap the microplate carrier for the test tube rack to dispense to test tubes with the MicroFlo:

- 3 Turn off the MicroFlo.
- 4 Optionally, disconnect the drain tube from the prime trough.
- 5 Remove the prime trough by sliding it towards you and away from the dispenser.
- 6 Move the plate carrier all the way to the left and lift it up and off its rails. Lift the carrier's left side first.



- 7 Place the test tube rack on the rails with the engraved FRONT label oriented to the front of the instrument. Notches in the base of the test tube rack secure it in place. Make sure the rack is level.
- 8 Reinstall the priming trough and drain tube.

❖ **Note:** Only four tubes are used to dispense to the test tube rack. Tubes 1, 3, 5, 7 must be removed from the fluid vessel before beginning a dispense.

Dispensing to the test tube rack

❖ **Important:** Read the Special Plates section in the Essential Concepts chapter before dispensing to test tubes.

The MicroFlo supports two types of test tubes in the test tube rack:

- 20 - 12 mm X 75 mm, 6 mL capacity tubes. Select **75T** when using the keypad.
- 20 - 13 mm X 100 mm, 9 mL capacity tubes. Select **100T** when using the keypad.

Before dispensing to a test tube rack, the odd-numbered dispense tubes must be removed from either the fluid supply or from the cassette itself. Only the even-numbered channels are properly positioned to dispense to the tubes.

❖ **Remove channels 1, 3, 5, and 7 from the cassette or fluid supply before dispensing to test tubes.**

| Plate Type | Columns/Rows | Tubes per Well | Tubes removed |
|-----------------|--------------|----------------|---------------|
| 20 75 mm Tubes | 5 x 4 | 1 | 1, 3, 5, 7 |
| 20 100 mm Tubes | 5 x 4 | 1 | 1, 3, 5, 7 |

When the rack is installed, and the unused tubes removed from the cassette or the fluid vessel, you can run a Quick Dispense or create a Protocol as you normally would. The MicroFlo will adjust the dispense height automatically when you select the correct plate type: 75T or 100T.

Appendix C

Gravimetric Calibration Instructions

This appendix provides an overview and step-by-step instructions for gravimetrically calibrating a cassette. This procedure can also be used to meet the monthly requirement to “Verify Performance.”

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Calibrating the Tubing Cassette Gravimetrically

To ensure precise and accurate dispensing, the tubing cassette must be calibrated. This procedure is one of several options for meeting this requirement. You can purchase calibrated cassettes from BioTek, rather than recalibrate a used cassette. Or, you can purchase a MicroFlo accessory from BioTek: the Calibration Kit, PN 7170017.

This procedure can also be used to meet the monthly requirement to “Verify Performance.”

This gravimetric method takes an experienced lab tech about 45-60 minutes to complete. It is an iterative process of measuring and adjusting until the desired accuracy is achieved.

Before you begin, learn “About Calibrating Cassettes Gravimetrically” on page 105.

These instructions are based on dispensing water to calibrate the tubing, which is sufficient for most applications. Adjust the formulas used in this method to accommodate a different test fluid, if desired.

❖ **Recommended:** Allow the cassette to equilibrate to ambient room conditions for at least 2 hours prior to calibration.

Required Materials:

- A supply vessel containing the deionized (DI) water, with a little dye, if desired, to make it easier to see the fluid.
- 96-well, 1x12 strip microplate shipped with the dispenser (Greiner Bio-One, Cat. No. 701070 or equivalent).
- Precision digital balance with a capacity of 1000 g and readability of 0.0001 g resolution. If unavailable, perform multiple dispenses to sufficiently increase the dispense weight.
- **MicroFlo Select.**
- **Gravimetric Cassette Calibration Worksheet:** two formats are offered, an Excel® worksheet is provided on the Operator’s Manual CD in the Spreadsheets folder and a tabular form can be found on page 104.



Figure 1: A MicroFlo Select in preparation for the gravimetric cassette calibration, and a digital precision balance with 0.0001 g resolution.

Procedure:


- 1 Install the cassette to be calibrated.
- 2 Turn on the MicroFlo Select and make sure the cassette type setting is correct.
- 3 Turn on the balance.
- 4 Define a Protocol with the following parameters:
 - Set the **Dispense Volume** to match the cassette:

| Cassette | Volume |
|------------------|-------------------|
| 1 μL | 10 μL |
| 5 μL | 50 μL |
| 10 μL | 100 μL |

- Set the **Flow Rate** to:
 - 1 μL cassette = Medium
 - 5 μL cassette = High
 - 10 μL cassette = High
- Retain the default settings for Dispense Height and Horizontal Position
- Define a **Prime before start**: set volume to 10 μL .
- Set the **Number of Pre-dispenses** to 2.



Figure 2: Measure and record the weight of each of the strips prior to dispensing.

 **Tip:** Label the strips with a letter for each row, 1 – 8, to make it easier to keep track of them when weighing each one individually.

- 5 Place each strip on the scale and record its mass (using the provided worksheets). This will be the **Tare** mass for each of the strips.
- 6 Put the tube organizer into the test fluid supply vessel.
- 7 **Prime** the tubing until any visible air bubbles are removed.
- 8 **Run** the dispense protocol.



Figure 3: Measure and record the weight of each of the strips after dispensing.

- 9 Measure and record the **Gross** mass (the mass of the strip and fluid) of each strip.

The worksheet subtracts the **Tare** mass from the **Gross** mass to obtain the **Actual** mass dispensed into each strip. It subtracts the **Expected** mass from the **Actual** mass. From these values, it displays a “**Correction**” recommendation for the number of turns of the screw and the current **Accuracy** percentage.

- 10 Depending on the result of (Actual mass - Expected mass), use the screwdriver shipped with the MicroFlo to adjust the tube’s tension screw:
- **Correction** (turns): the **Gravimetric Cassette Calibration Worksheet** displays a recommended number of turns. Turn clockwise for a positive number; turn counter-clockwise for a negative number.

(Actual – Expected) = Positive Value

When the difference between the expected and the actual mass is positive (too much fluid dispensed) turn the adjustment screw **clockwise** to increase tension on the tubing.

(Actual – Expected) = Negative Value

When the difference between expected and actual mass is negative (not enough fluid dispensed) turn the screw **counter clockwise** to decrease tension.



Figure 4: Use the screwdriver shipped with the MicroFlo to adjust the tension in the tubing.

- 11 Repeat the process, beginning at Step 5, until the desired Accuracy % is achieved.

Gravimetric Cassette Calibration Worksheet

Cassette Type: _____ (1μL, 5μL, 10μL) Correction Factor (k): _____

Density (ρ): _____ (g/μL) Number of Wells: _____

Volume per well (V): _____ (μL) Number of Runs: _____

Expected Mass: _____ (g) $m_{\text{expected}} = \rho \cdot V \cdot (\text{No. of Wells}) \cdot (\text{No. of Runs})$

Accuracy: _____ % $\text{Accuracy (\%)} = 100 \frac{(m_{\text{actual}} - m_{\text{expected}})}{m_{\text{expected}}}$

| | | Dispensed Mass | | | Actual Mass - Expected Mass (g) | Correction (turns) | Accuracy (%) |
|---------|--------------|----------------|--------------|---------------|---------------------------------------|-----------------------|-----------------|
| | | Tare (g) | Gross (g) | Actual (g) | | | |
| Trial 1 | 1 x 12 Strip | A | | | | | |
| | | B | | | | | |
| | | C | | | | | |
| | | D | | | | | |
| | | E | | | | | |
| | | F | | | | | |
| | | G | | | | | |
| | | H | | | | | |

| | | Dispensed Mass | | | Actual Mass - Expected Mass (g) | Correction (turns) | Accuracy (%) |
|---------|--------------|----------------|--------------|---------------|---------------------------------------|-----------------------|-----------------|
| | | Tare (g) | Gross (g) | Actual (g) | | | |
| Trial 2 | 1 x 12 Strip | A | | | | | |
| | | B | | | | | |
| | | C | | | | | |
| | | D | | | | | |
| | | E | | | | | |
| | | F | | | | | |
| | | G | | | | | |
| | | H | | | | | |

| | | Dispensed Mass | | | Actual Mass - Expected Mass (g) | Correction (turns) | Accuracy (%) |
|---------|--------------|----------------|--------------|---------------|---------------------------------------|-----------------------|-----------------|
| | | Tare (g) | Gross (g) | Actual (g) | | | |
| Trial 3 | 1 x 12 Strip | A | | | | | |
| | | B | | | | | |
| | | C | | | | | |
| | | D | | | | | |
| | | E | | | | | |
| | | F | | | | | |
| | | G | | | | | |
| | | H | | | | | |

About Calibrating Cassettes Gravimetrically

The gravimetric calibration method uses known values (the expected mass of a volume of water/fluid) to set the accuracy of each dispense tube. BioTek ships a 12 X 8 strip plate with the MicroFlo for this purpose. In this procedure, each strip is tared before dispensing and weighted again after dispensing. When a tube dispenses too much or too little fluid, it must be calibrated.

Tubes are calibrated by adjusting the tension screw in the cassette's tube tensioner. A clockwise rotation, \cup , of the adjustment screw increases tension on the tubing and reduces the volume of fluid dispensed. A counter clockwise rotation, \cup , decreases the tension and increases the volume dispensed.

Calibration is required for a cassette to meet the MicroFlo's specifications.

Determining Expected Mass

The expected mass (m_{expected}) of the fluid in the strip is the product of the density of the fluid (ρ), the volume of fluid dispensed into each well (V), and the number of wells being dispensed to and the number of runs performed:

$$m_{\text{expected}} = \rho \cdot V \cdot (\text{No. of Wells}) \cdot (\text{No. of Runs})$$

BioTek calibrates the cassettes using deionized water. The density of water at standard temperature and pressure is 0.0010 g/ μL , and the number of wells on a single 1 x 12 strip is 12, so if we dispense to the plate once (1 run), the equation above becomes:

$$m_{\text{expected}} = 0.0120 \cdot V$$

This table shows the expected mass for various dispense volumes:

| Volume | Expected Mass |
|-------------------|---------------|
| (μL) | (g) |
| 1 | 0.0120 |
| 2 | 0.0240 |
| 5 | 0.0600 |
| 10 | 0.1200 |
| 20 | 0.2400 |
| 50 | 0.6000 |
| 100 | 1.2000 |

You can determine similar values for the fluid type and dispense volumes regularly used in your lab, if desired. The MicroFlo's performance at small volumes may be improved by calibrating the cassette with the specific fluid to be dispensed.

Determining the Amount of Rotation

The amount of fluid dispensed may be adjusted by increasing or decreasing the tension in the tubing. A clockwise rotation of the adjustment screw increases tension on the tubing and reduces the volume of fluid dispensed. A counter clockwise rotation decreases the tension and increases the volume dispensed.

The amount of rotation, or number of turns of the screwdriver, required to change the mass of fluid dispensed can be estimated as:

$$\text{Turns} = k \frac{(m_{\text{actual}} - m_{\text{expected}})}{m_{\text{expected}}}$$

Where $m_{\text{actual}} - m_{\text{expected}}$ is the difference between the actual mass and the expected mass, and an experimentally derived calibration constant, k , for each of the cassettes which is approximately:

- 1µL Cassette: $k = 60.7$ turns
- 5µL Cassette: $k = 51.3$ turns
- 10µL Cassette: $k = 42.6$ turns

BioTek provides the **Gravimetric Cassette Calibration Worksheet** to facilitate the calibration procedure. This Excel® spreadsheet uses this formula to recommend the direction and number of turns of the screw required to calibrate the tube.

Accuracy Specifications

The accuracy specifications for the cassettes are intended to describe the performance of tubing throughout its life. Typically, achieving a dispense accuracy that is 1/10th of the specified accuracy is the goal of calibration.

Dispense accuracy is computed using this formula:

$$\text{Accuracy (\%)} = 100 \frac{(m_{\text{actual}} - m_{\text{expected}})}{m_{\text{expected}}}$$

Guidelines for Adjusting the Tension Screw

It is a trial and error process determining the correct position of a tube's tension screw. BioTek developed these guidelines to help, but hands-on experience is the only way to become proficient at it.

- To give you a feel for the effect of the tension screws, this how a ½ turn of the screw alters the tube's volume:

| Cassette | Volume | Difference |
|----------|--------|------------|
| 1 µL | 10 µL | .08 µL |
| 5 µL | 50 µL | .5 µL |
| 10 µL | 100 µL | 1 µL |

- ☺ Turn the screw clockwise to reduce the dispense volume.
☹ Turn the screw counter-clockwise to increase the dispense volume.

- Autoclaving the cassette may change (increase) the tubing's capacity. After multiple autoclave cycles, recalibration may be required.
- When working with a newly built cassette, use the numbered scale on the Tube Tensioner's face to evenly line up the blocks. Calibration will be easier when all the tubes begin at the same point.

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