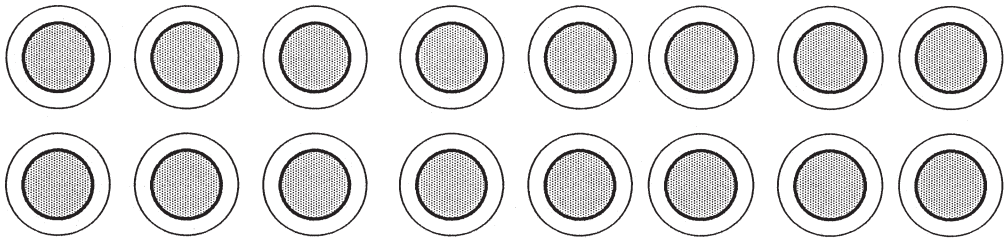
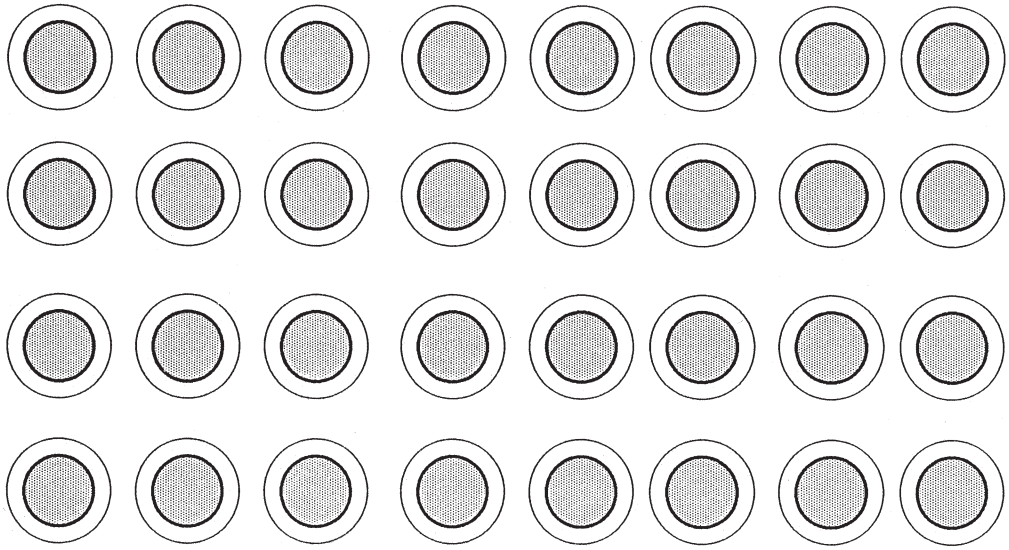


EL404

Microplate Washer

User's Guide



BIO-TEK® INSTRUMENTS, INC.

EL404 Manual Update

Rev. G1 P/N 4101000

The information in this update pertains to page *3-11* of Chapter 3, in the EL404 User's Guide.

Creating and Editing a Wash Program

In certain situations, changes made to the soak time may not be retained in system memory. The following note should be added to Step 6:

> **Note:** Due to software architecture, a change in soak time is retained in system memory only when another parameter is changed at the same time. Therefore, to permanently change the soak time from 020 to 030, you should simultaneously change the wash volume from 300 to 350 (or any imaginary number you choose). Then exit the wash program, enter it again, and re-enter the correct wash volume.

EL404 User's Guide

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BIO-TEK Part No. 4101000

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Revision Appendix

Revision	Date	Page	Change
A	02/94	--	
B	03/94	Document	Text changes throughout to improve readability
C	08/94	Preface, document	Correct chapter references in TOC, document
D	03/95	Appendix A, document	Added Appendix A, Computer control; altered references to computer control throughout.
E	9/19/95		Add Notice to manual
F	10/20/95		Incorporate notice info in manual
G	01/30/96		Changed Appendix A

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EL404 Intended Use Statement

The EL404 is an Automated Microplate Washer which provides microplate priming, washing and non-critical dispensing for ELISA, fluorescence and bioluminescence immunoassays, cellular and agglutination asays. It can also be used to dispense reagent where dispensing precision and reagent dead volume are not critical.

PREFACE

Welcome to the EL404

This User's Guide is your introduction to Bio-Tek's EL404 Automated Microplate Washer. Use it now to learn more about how the EL404 works and to begin operating the microplate washer. Use it later as a reference manual for your daily operations and as a guide for trouble-shooting.

How to Use this Guide

This guide is organized into 7 chapters and an Appendix. A Short Form Instructions section may be included to help those familiar with Bio-Tek's family of Automated Microplate Washers begin operation immediately.

Chapter 1, "Features and Functions," provides a brief explanation of the EL404's features, and a summary of the EL404's components and functionality. It explains —

- New features
- Functionality of the washer
- An Isometric view of the EL404, and a discussion of its major components

Chapter 2, "Installing the EL404," describes unpacking and setup of the EL404. including bottle and tubing setup. The chapter describes how to —

- unpack and inspect the EL404
- attach the microplate carrier
- set up bottles and tubes for Pressure, Combined Pressure/Vacuum, Vacuum pump and Gravity feed operations

Chapter 3, "Operation" takes a focused view of the EL404's major function: washing a microplate. The chapter details —

- The EL404 keyboard
- Loading a microplate
- Setting up a wash program
- Microplate height adjustment
- Dispense overflow height adjustment
- Priming the washer
- Routine microplate washing

Chapter 4, "Functions: Dispense, Aspirate and Rinse," provides comprehensive information on the additional functions of dispensing, aspiration and rinsing. It explains —

- How to dispense fluid
- How to aspirate fluid from microplate wells
- Rinsing the microplate

Chapter 5, " Maintenance," describes the regular maintenance that should be performed on the EL404, including —

- Daily maintenance
- Weekly maintenance
- Monthly maintenance
- Decontamination
- Fuse replacement

Chapter 6, "Resolving Problems and Support" includes —

- How and when to contact Customer Service
- Error Messages
- Troubleshooting Scenarios

Chapter 7, "Reference," provides information on —

- EL404 specifications
- Optional Accessories and ordering information
- Requirements for user-supplied accessories

Appendix A, "Computer Control" explains —

- How to use a computer to control the operation of the EL404.

Notational Conventions

A number of notational conventions are followed throughout this guide; these are designed to draw your attention to special information.

Special information is called out with the following symbols, usually in a sidebar format.

Warning: Safety information is presented in this format. Please read these notices carefully.

Precautions to take to avoid system damage are presented in this format.

Helpful information about problems that may arise is presented in this format.

Cross-referenced information is presented in this format.

Numbered Steps:

Whenever you must perform a procedure with a number of steps they are displayed as below. Perform each step completely, and in the order presented.

Sometimes these steps are accompanied by explanatory text. This will appear immediately after the numbered step and will explain more about the operation.

1. **This is an example of a numbered step.**

   This is where you will find explanatory text.

Warnings and Precautions

When operated in a safe environment, according to the instructions in this manual, there are no known hazards associated with the EL404. However, the operator should be aware of certain situations which could result in serious injury.

Hazard Warnings

Warning! Power Rating. the EL404 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 EL404. Use of a two-prong adapter disconnects the utility ground, creating a severe shock hazard. Always connect the system power cord directly to a three-prong receptacle with a functional ground.

Warning! Internal Voltage. Always turn off the power switch and unplug the power cord before cleaning the outer surfaces of the instrument.

Warning! Liquids. Avoid spilling liquids on the instrument; fluid seepage into internal components creates a potential shock hazard. Wipe up all spills immediately. Do not operate the instrument if internal components have been exposed to fluid.

Warning! Shock. Overpressurizing the unit can cause fluid leakage interally, creating a potential shock hazard.

Precautions

The following precautions are provided to help you avoid damaging the system:

Caution: Service. The system should be serviced by Bio-Tek-authorized service personnel. Only qualified technical personnel should perform troubleshooting and service procedures on internal components.

Caution: Replacement Fuses. Use replacement fuses with the required current rating and specification. Improper fuses or short-circuiting the fuse holders may cause fire or damage the instrument.

Caution: Environmental Conditions. Do not expose the system to temperature extremes. System performance may be adversely affected.

PREFACE

Caution: Sodium Hypochlorite. Do not expose any part of the instrument to Sodium Hypochlorite solution (bleach) for more than 30 minutes. Prolonged contract may damage the instrument surfaces. Be certain to rinse and thoroughly wipe all surfaces.

Caution: Although many precautions have been taken to ensure that the instrument is as corrosion proof as possible, Ensure that spillage of wash buffer solution is wiped; prolonged exposure to salt solution without cleanup can lead to corrosion of the microplate carrier and movement rails.

Electromagnetic Interference and Susceptibility

USA FCC CLASS A

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

Note: This equipment has been tested and found to comply with the limits for a Class A digital device, pursuent 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 harmful interference in which case the user will be required to correct the interference at his 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'met 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.

EC EN 55011 GROUP 1, CLASS A - EC DIRECTIVE 89/336/EEC

The system has been type tested by an independent testing laboratory and found to meet the requirements of EC Directive 89/336/EEC and also VDE 0871/CISPR 11:1990 for Radiated Emissions, Line Conducted Emissions, and the Low-Frequency Magnetic Field Test. Verification was to the limits and methods of EN 55011 and VDE 0876/0877. The device is classified as EN 55011 Group 1, Class A.

EC EN 50082-1

The system was also tested and found to meet requirements for Electrostatic Discharge Susceptibility, Radiated Susceptibility, and Conducted Susceptibility. Verification of compliance was conducted to the limits and methods of EN 50082-1:1992; IEC 801-2:1984; IEC-801-3:1984; and 801-4:1988.

Safety

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

Underwriters Laboratories Standard UL UL1262 Laboratory Equipment;

Canadian Standards Association C22.2 No. 151-M1986 Laboratory Equipment; and

IEC 1010-1 (1990) "Safety requirement for electrical equipment for measurement, control and laboratory use. Part 1 General requirements."

Safety Symbols

The following warning and informational symbols may be found in various locations on the EL404. The warnings are given here in English, French, German, Spanish and Italian. Only qualified personnel who recognize shock hazards and are familiar with the safety precautions should use this instrument. read the manual carefully before operating this instrument.

Direct current
Courant continue
Gleichstrom
Corriente continua
Corrente continua

Alternating current
Courant alternatif
Dreiphasen-Wechselstrom
Corriente Atterna
Corrente alternata

Both direct and alternating current
Courant continu et courant alternatif
Allstrom (Gleich - und Wechselstrom
Corriente continua y corriente alterna
Corrente continua e corrente alternata

Earth ground terminal
Borne de terre
Erde (Bettriebserde)
Borne de Tierra
Terra (di funzionamento)

Protective conductor terminal
Borne de terre de protection
Schutzleiteranscluss
Borne de Tierra de Protección
Terra di protezione

On (Supply)
Marche (alimentation)
Ein (Verbindung mit dem Netz)
Connectado
Chiuso

Off (Supply)
Arrest (alimentation)
Aus (Trennung vom Netz)
Desconectado
Aperto (sconnessione dalla rete di alimentazione)

Caution (refer to accompanying documents)
Attention (voir documents d'accompanement)
Achtung siehe Begleitpapiere
Atención (vease los documentos incluidos
Attenzione, consultare la doc annessa

Caution, risk of electric shock
Attention, risque de choc electrique
Gefährliche elektrische Spannung
Atención, riesgo de sacudida eléctrica
Alta tensione (in questo documento Alta tensione non significa "tensione pericolosa" come definito in IEC 417)

What's Next

The next chapter, "Features and Function" provides a brief explanation of the EL404's features, and a summary of the EL404's components and functionality.

CHAPTER 1

Features and Function

This chapter provides a brief explanation of the EL404's features, and a summary of the instrument's functuality. The chapter also includes —

- An isometric view of the EL404, and a discussion of its major components, and
- a brief walkthrough of EL404 operations.

About the EL404

Bio-Tek's newest Automated Microplate Washer, the EL404 has been designed to make microplate washing as simple as possible for the user. The instrument's easy to read displays and precision software guides the user through each step of microplate priming, washing and maintenance.

The EL404 automatically removes unbound reagents in 96-well microplates in immunological assays such as Enzyme Linked Immunosorbent Assays (ELISA), fluorescence immunoabsorbant assays (FIA), and chemiluminescence assays. In addition, the instrument may also be used for many types of cellular and agglutination assays.

The EL404 can also be used to dispense solutions when pipetting precision is not necessary. Front panel controls enable the user to select fluid volume, soaking time, number of wash cycles, aspiration depth, shake time and frequency, and program name. Safety features include signaling dispensing and aspiration malfunctions and overflow protection. The EL404 surfaces can be wiped clean.

Features

The EL404 incorporates the following features into its design:

- **Display:** An enlarged display (2 x 24) that allows the entire wash program to be simultaneously viewed on the display, instead of relying on a nested menu structure. As each wash variable is programmed, the variable is described at the top of the display.

- **Washing:** Up to 10 different wash programs may be selected, allowing simultaneous washing of all 96 wells in 8 seconds. The

user may specify the number of wash cycles, fill volumes, soaking duration, shaking, and whether to leave the microwells empty or filled.

- **Edit Program Name:** Each wash program may be named according to the title of the actual assay that is being processed; i.e. naming a program "Hepatitis" enables easier identification of the program than if were just referenced by a Program number.

- **Software Height Adjustment:** The software height adjustment range of 0-200 allows the accommodation of a wide variety of microplates with a high degree of precision, reliability and consistency from washer to washer. Values programmed into one washer can be easily transferred to another, assuring easy microplate setup.

- **Shaking:** Shaking frequencies and time selections have been expanded to accommodate a greater variety of shaking applications; i.e. some cellular assays might require slower shaking and other assays a variety of shaking time and speed.

- **Empty and Prime Routine:** In addition to the standard Prime function, the Empty and Prime routine combines emptying the manifold and the inside fluid line, with the Prime routine. This new easy-to-use routine attains almost perfect purity on the first prime, while avoiding the need for a second prime.

- **Service Mode:** The new service mode allows manual manipulation of valves enabling the user to test their function.

- **Computer Control:** Computer control operations are available, via the EL 404's RS232 port.

- **Error Messages:** Precise, easy-to-understand error messages describe the problem, rather than using numerical or universal error flags.

- **Aspiration:** Top-down aspiration that does not immerse the tubes in the microplate well, thus minimizing carryover from one microplate to another;

- **Robotics Interface**: The microplate carrier has automatic lift mechanism that is oriented to facilitate a robotics interface;

- **Shield:** The removable aerosol shield isolates the microplate bring washed from the laboratory environment.

A Brief Walkthrough

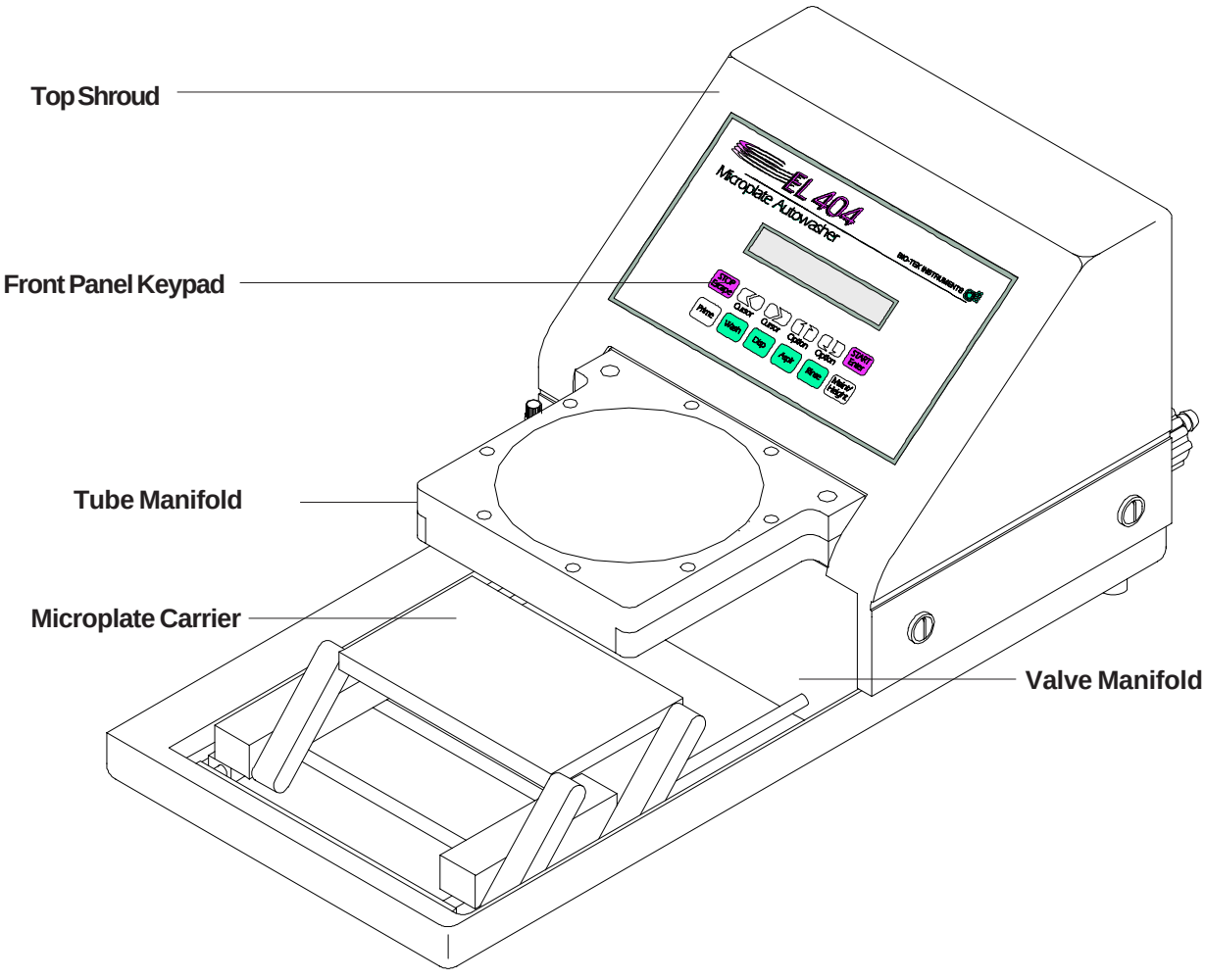
Chapter 3, “Operation,” provides a detailed description of the EL404’s operation. This section provides a brief walkthrough of the EL404’s major components and their functions.

Summary of EL404 Components and Functionality

The EL404 can handle simultaneous washing of 96 microplate wells in using 10 different user-customized wash programs. The major components include microprocessor controls, a tube manifold for the dispensing chamber and aspiration channels, a valve manifold and a flow sensor to regulate the dispensing.

The system also includes a microplate carrier mechanism, and a variety of motors and pumps to move plates and fluids.

The EL404 starts its wash cycle — removing the contents of the microplate — by opening the aspiration valve to the vacuum source. Once empty, the wells are filled to a preset volume by opening the dispense valve to fluid from the gravity or pressurized reservoirs. Opening the aspiration valve to the vacuum source completes the wash cycle by removing the contents of each well. This basic operation and all diagnostic functions are controlled by the microprocessor.



Microprocessor

The microprocessor controls all functions of the EL404. It interprets signals from the flow sensor, controls the actions of the valves on the valve manifold, and performs self-diagnostic tests for each of its functions.

Tube Manifold

The tube manifold, which holds the aspiration and dispense tubes, is located on the front of the EL404 underneath the front control panel.

The tube manifold is actually a top and base sandwiched together to form an inner dispensing chamber and 12 aspirating channels. The top piece is clear acrylic (to allow the operator to observe the flow of liquid) and the bottom piece is constructed of a chemically resistant polyvinylchloride (CPVC).

The short dispense tubes descend from the fluid reservoir past the aspiration channels into the microplate wells. The longer aspirate tubes (which originate in the aspiration channels contained within the bottom piece of the tube manifold) descend into the microplate wells.

Valve Manifold

The valve manifold, located beneath the rear of the tube manifold, is controlled by the microprocessor to direct the flow into and out of the tube manifold. Constructed of black acetal, this manifold contains four solenoid valves which control fluid flow into and out of the tube manifold. The four valves are:

- **Dispense valve:** The dispense valve controls all fluid flowing into the system. The wash, prime, rinse, and dispense functions open this valve, and allow fluid from the fluid reservoirs to flow from the valve manifold into the tube manifold.

When the EL404 is connected to a vacuum source, the aspirate, purge, and air-bleed valves control all fluid removal from the instrument.

- **Purge valve:** The purge valve, only open during rinse or overnight maintenance, allows removal of residual fluid from the entire tube manifold.

- **Aspirate valve:** All functions use the aspirate valve. The aspirate valve opens the long aspiration tubes to vacuum so that the fluid in the microplate wells can be suctioned out.

- **Air-bleed valve:** The air-bleed valve, used to remove air from the dispense portion of the manifold, is opened during any prime. The air-bleed valve opens to allow air bubbles to escape.

Flow Sensor

The flow sensor monitors the fluid flow in the EL404, enabling the microprocessor to adjust flow by opening and closing the valves in the valve manifold. If the sensor determines that the flow is too weak, a warning tone sounds, and an error message appears on the display.

Microplate Carrier

The microplate carrier holds the microplate in place. It is made of corrosion-resistant aluminum and stainless steel and is designed to hold flat, *U*-(round), and *V*-bottom microplates. The carrier is easily removed for cleaning.

Front Panel Keypad

The front panel keypad is described in detail in Chapter 2.

Vacuum sensing switch

The vacuum sensing switch, located on the lower right-hand corner of the EL404's back panel, senses the presence of vacuum to the EL404. The washer will not perform any function unless tubing connects the switch to adequate vacuum.

Aerosol cover

A clear plastic aerosol cover is designed to isolate the microplate undergoing washing from the surrounding laboratory environment. The cover can be easily removed by loosening the 4 cover retaining screws and lifting the cover off of the washer.

Drip pan

The drip pan is used to catch occasional drops from the dispense tubes when the EL404 is sitting idle and the microplate carrier is ejected.

Mirror

The mirror is used to observe the dispensing and aspirating process through the bottom of the microplate carrier.

Rear Panel

The following major components are located on the rear panel of the washer:

Power Module:

The Power Module can be found on the upper left corner of the washer's rear panel. The Power Module contains:

- **On/Off Switch:** Located at the top of the power module, this switch provides power to the EL404.

- **Input receptacle**: Three pronged.

- **Fuse Compartment.** Contains one 230V fuse. When the fuse compartment cover is pried off, the fuse holder is exposed. Refer to Chapter 6 for fuse replacement instructions.

Dispense Fluid In:

This port allows fluid from the fluid reservoir into the washer.

Main Vacuum Out:

This port allows vacuum generated by the aspiration setup to suction air and fluid out of the washer.

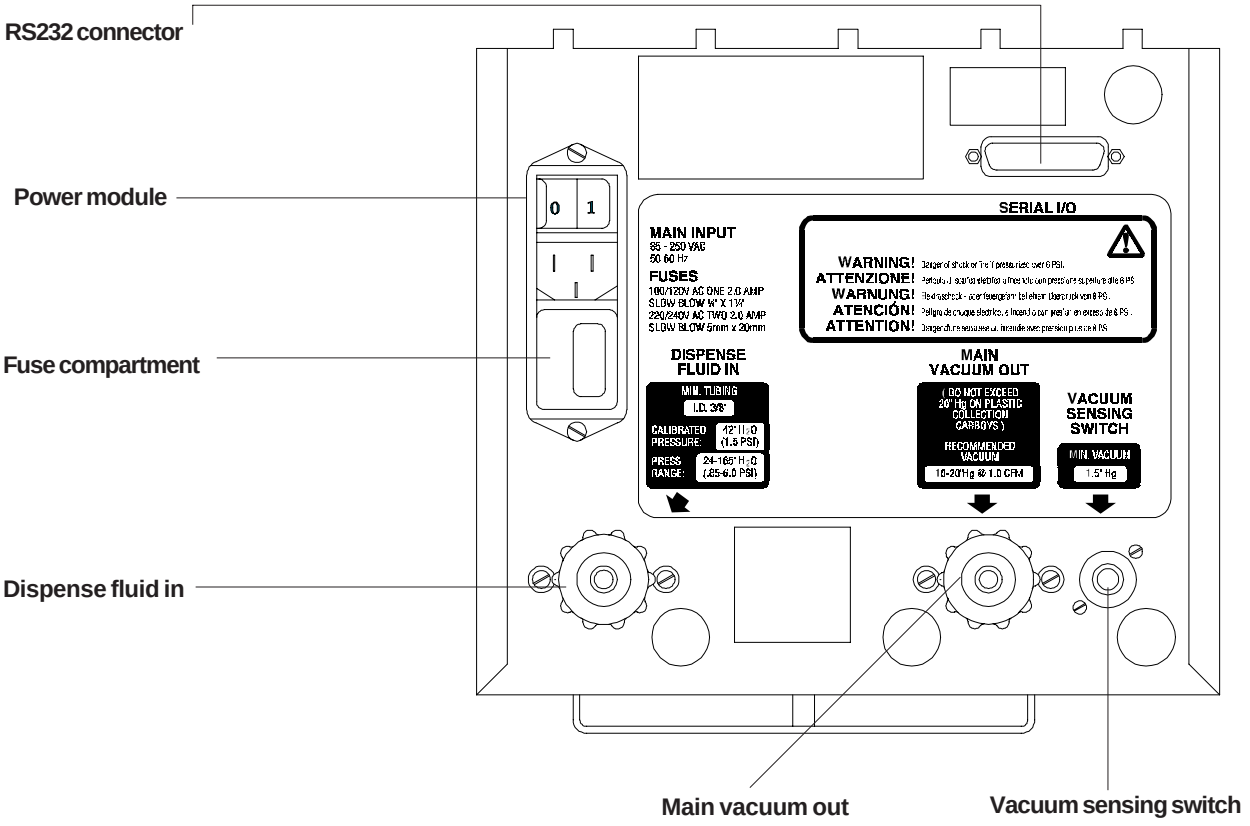


Figure 1-2: *EL404 Rear Panel*

Vacuum Sensing Switch:

This switch monitors the vacuum source.

RS232 Port

This 25-pin Serial port allows for computer control of the washer. This option is discussed in Appendix A.

What's Next

The next chapter, "Installing the EL404" describes unpacking and setup of the washer, including attaching the microplate carrier, setting up bottles and tubes for Pressure, Combined Pressure and Vacuum, Vacuum Pump and Gravity Feed operations.

CHAPTER 2

Installing the EL404

This chapter describes unpacking and setup of the EL404, including configurations for bottles and tubing. The chapter details how to —

- Unpack and inspect the EL404
- Attach the microplate carrier
- Set up bottles and tubes for Pressure, Combined Pressure/ Vacuum, Vacuum Pump and Gravity Feed operations
- Prime the tubing

Unpacking and Placement

Unpack the EL404 and accessories from the shipping carton, and inspect the washer carefully for damage, such as cracks, dents or bent parts. If you find any physical damage, or if the EL404 does not operate correctly when powered up, please call Bio-Tek for assistance and notify the carrier if the damage appears to be the result of a shipping mishap.

Save the packing, which includes the box and foam. You must use the original packing materials if the instrument is returned to Bio-Tek at a later date. Repack the box and store it. The microplate carrier is stored in a compartment in the top foam piece.

Place the washer on a level working surface; this is important for maximum residual removal.

Accessories

The EL404 may be equipped with accessories, which will be listed on the packing slip. The washer should have a stylus and an Operator's Manual.

Dispense Accessories

A full set of dispense accessories consists of:

- One pressure pump or combined pressure/vacuum module
- Two 4-liter bottles with quick disconnects attached

- Two sections of thin tubing and one long piece of thick tubing with valves
- Four large white clamps and two small white clamps

Gravity Feed Accessories

A full set of gravity feed accessories consists of:

- Two 5-gallon bottles with spigots attached
- One set of long tubing with two valves and a Y-connector

Vacuum Accessories

A full set of vacuum accessories consists of:

- One vacuum pump or combined pressure/vacuum module
- Two 4-liter bottles with quick disconnects attached
- A set of two attached pieces of thick tubing and a separate piece of thick tubing
- Four large white clamps and one small white clamp

About the Microplate Carrier

The illustration below provides a detailed view of the EL404 microplate carrier. The microplate carrier has two adapter brackets to ensure the microplates fit securely in the carrier's microplate bed.

An additional adapter is needed if round-bottomed microplates are used. The adapter is stored on the left side of the carrier.

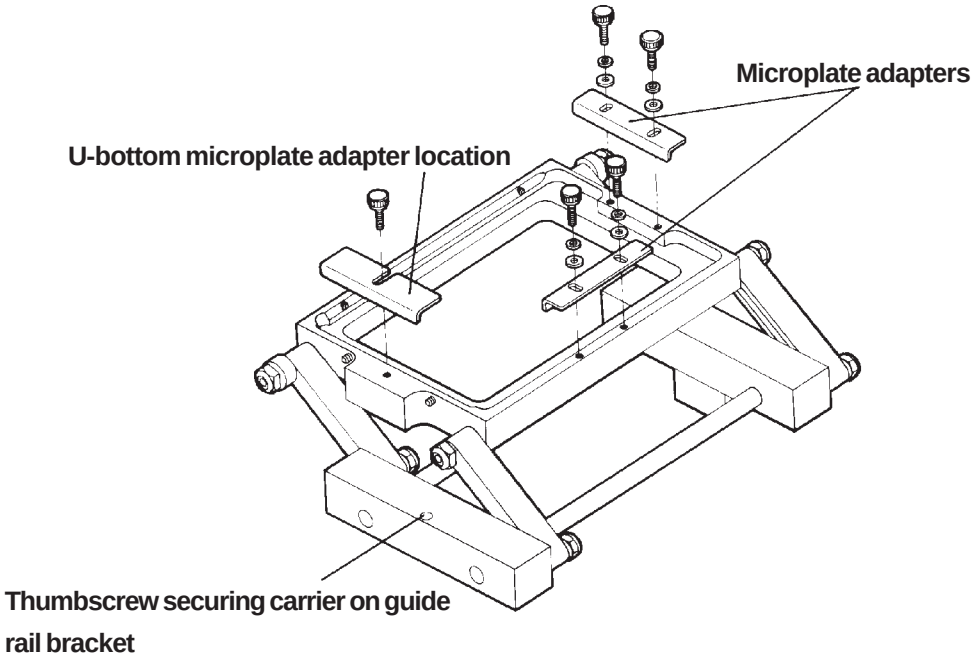


Figure 2-1. *Standard Microplate Carrier*

Attaching the Microplate Carrier

Use the illustration that follows to help you orient the microplate carrier and the EL404.

1. **Lower the guide rail slot on the microplate carrier onto the right guide rail of the EL404.**

2. **Gently lower the left side of the carrier onto the left guide rail on the EL404.**

   Use the two vertical carrier alignment pins to properly position the carrier on the guide rails.

3. **Rotate the thumbscrew on the left side of the microplate carrier to secure the carrier on the guide rail bracket.**

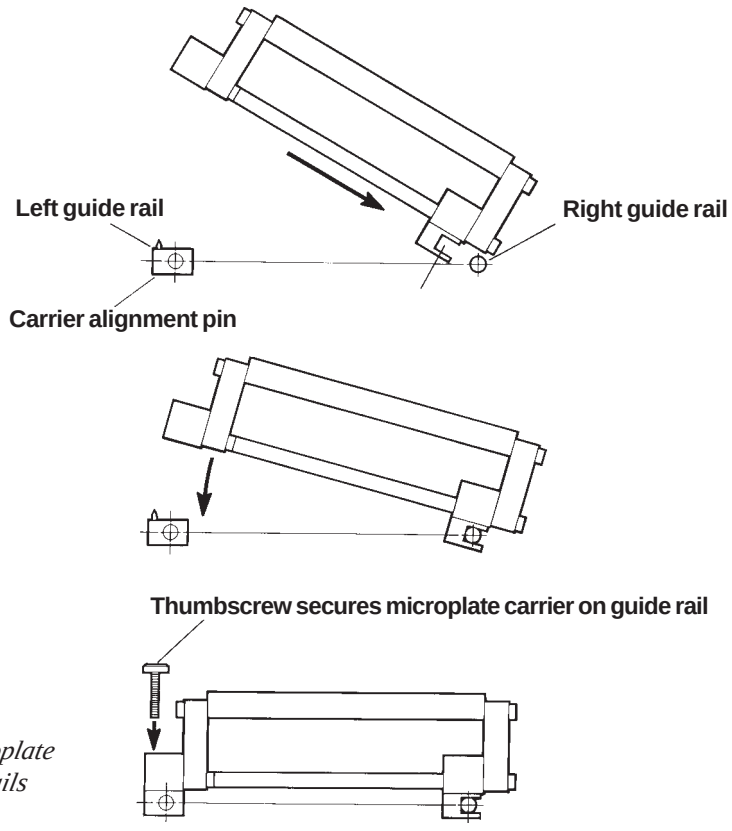


Figure 2-2: *Aligning the microplate carrier and the EL404 guide rails*

Bottle and Tubing Setup

Pressure, Combined Pressure/Vacuum Pump

1. Identify the two Dispense and two Vacuum bottles by the labels on the bottle caps.

2. Designate the four bottles as follows:

- Dispense bottle 1: Wash buffer
- Dispense bottle 2: Rinse solution
- Vacuum bottle 1: Primary collection bottle
- Vacuum bottle 2: Bubble trap to protect the vacuum pump.

The wash fluid can be phosphate-buffered saline with Tween, 0.9% saline, etc. Rinse fluid is usually deionized or distilled water.

3. Rinse the bottles.

4. Place the bottles, stands and the pumps on a level surface.

Customary placements include placing the pressure pump (pressure/vacuum module) and bottles to the right of the washer, and the vacuum pump and bottles to the left of the

washer. The pumps and bottles can also be placed on the floor. Metal stands are provided to help stabilize the bottles.

5. **Remove the caps and any plastic bags containing plastic disconnects.**

6. **Fill the dispense bottles, attach the caps and tighten them to prevent leaks.**

7. **Insert the male connectors into the IN and OUT ports of the bottles.**

8. **Attach the tubing by following the color coding on the tubing and bottles, and the diagrams on the following pages.**

   After setup, tubing can be cut to eliminate unnecessary length. reattach the color codes.

9. **The dispense bottles are pressurized in parallel by the pump via narrow 1/4" tubing. Connect the narrow end of the small tubing to the small port(s) (color-coded purple) on the front of the pump. Connect the wider end of the small tubing to the male connectors of the bottle ports (color coded purple).**

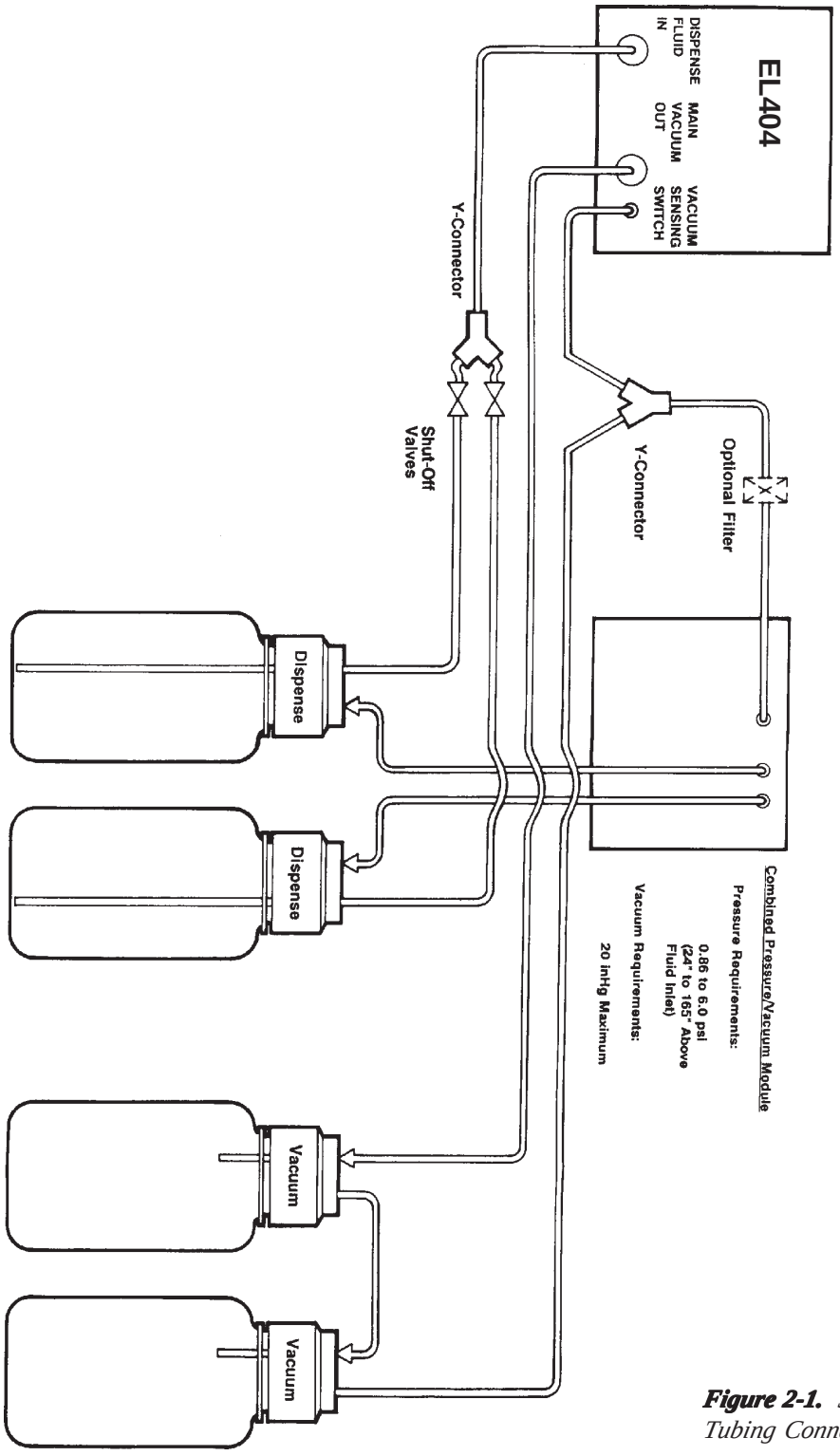


Figure 2-1. *System Setup Showing Tubing Connections*

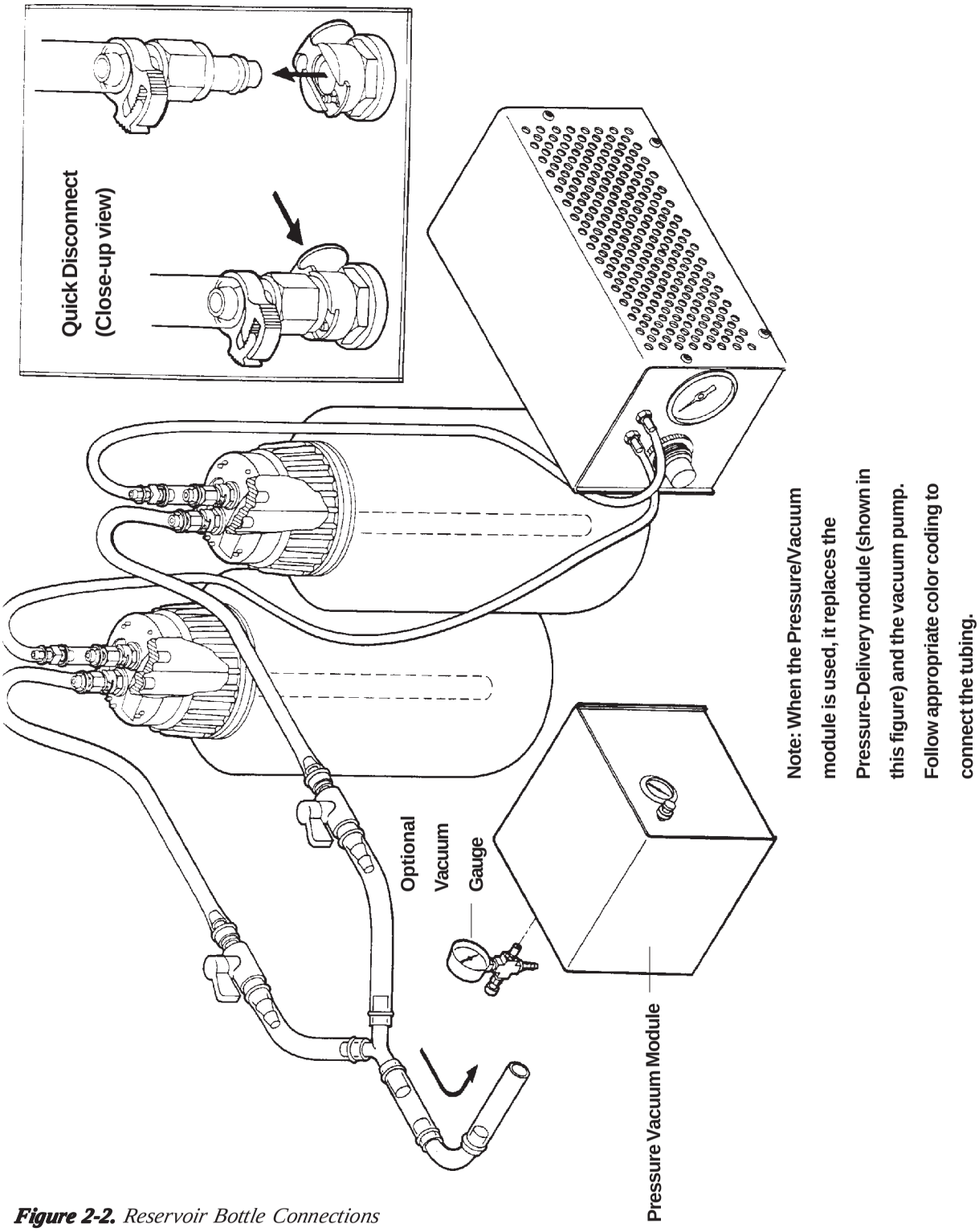


Figure 2-2. *Reservoir Bottle Connections*

10. **Connect the two (2) free ends (coded red and blue) of the large tubing with the Y connector and two valves (or 3-way valve) to the dispense bottle parts marked OUT.**

    The end with the Y-connector (or 3-way valve) connects with the washer port marked DISPENSE FLUID IN.

11. **The vacuum bottles are connected in series. Attach the orange Y-connector end of the long vacuum tubing to the vacuum pump.**

12. **Connect the larger and shorter tube (3/8" ID), which is attached to the Y connector, to the yellow port marked OUT on the bubble trap bottle.**

13. **Connect the free end of the long narrow tubing (the yellow coded 1/4" ID tubing attached to the Y-connector) to the recessed vacuum sensing switch on the lower right of the washer's rear panel.**

14. **Using the free, short piece of tubing, connect the port marked OUT on the primary collection bottle (color coded yellow) to the port marked IN (color coded green) on the bubble trap.**

15. **Connect one green labeled end of the remaining large-diameter tubing to the back of the EL404 and the other green-labeled end to the green-labeled port marked OUT on the bubble top.**

16. **Place the two small white clamps on the pressure delivery pump tubing connections, and the 8 large clamps on the dispense and vacuum connections.**

    Do not use the remaining mid-sized clamp.

17. **To disconnect the tubing, press the metal clip on the bottle top connectors.**

18. **If you are using the optional vacuum gauge supplied by Bio-Tek, plug the gauge into the main vacuum inlet port of the pressure / vacuum module. The gauge should have a male disconnect that plugs into the receptacle of the port.**

    The gauge is used to reduce the vacuum exerted by the module on the washer. This is useful for assays that are sensitive to vacuum force.

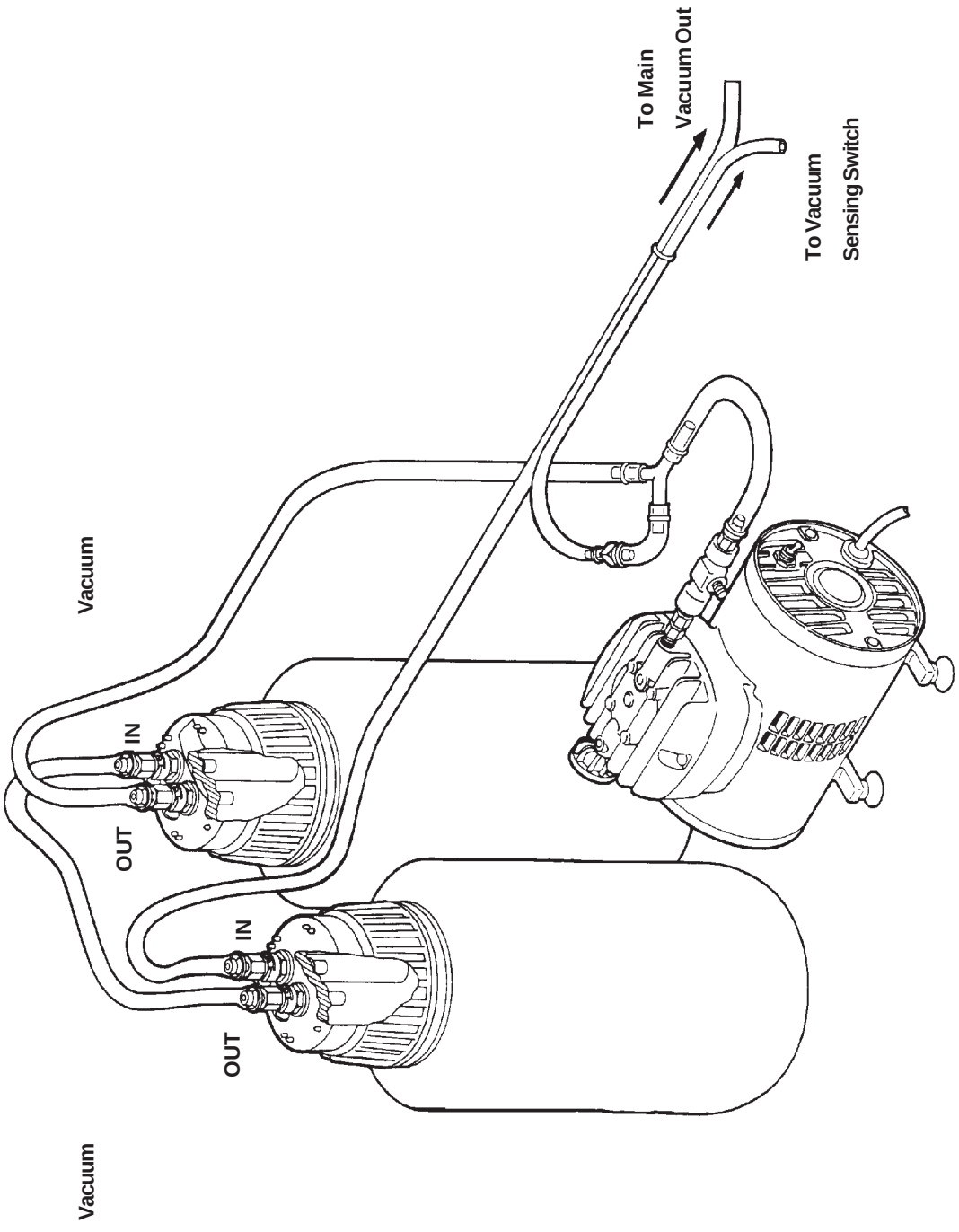


Figure 2-3: *Vacuum Pump Connections*

Gravity Feed Setup

1. **Designate 1 bottle for wash buffer and 1 for rinse solution.**

   Wash fluid can be phosphate-buffered saline with Tween, 0.9% saline, etc. Rinse fluid is usually deionized or distilled water.

2. **Rinse the bottles to remove any particulate matter.**

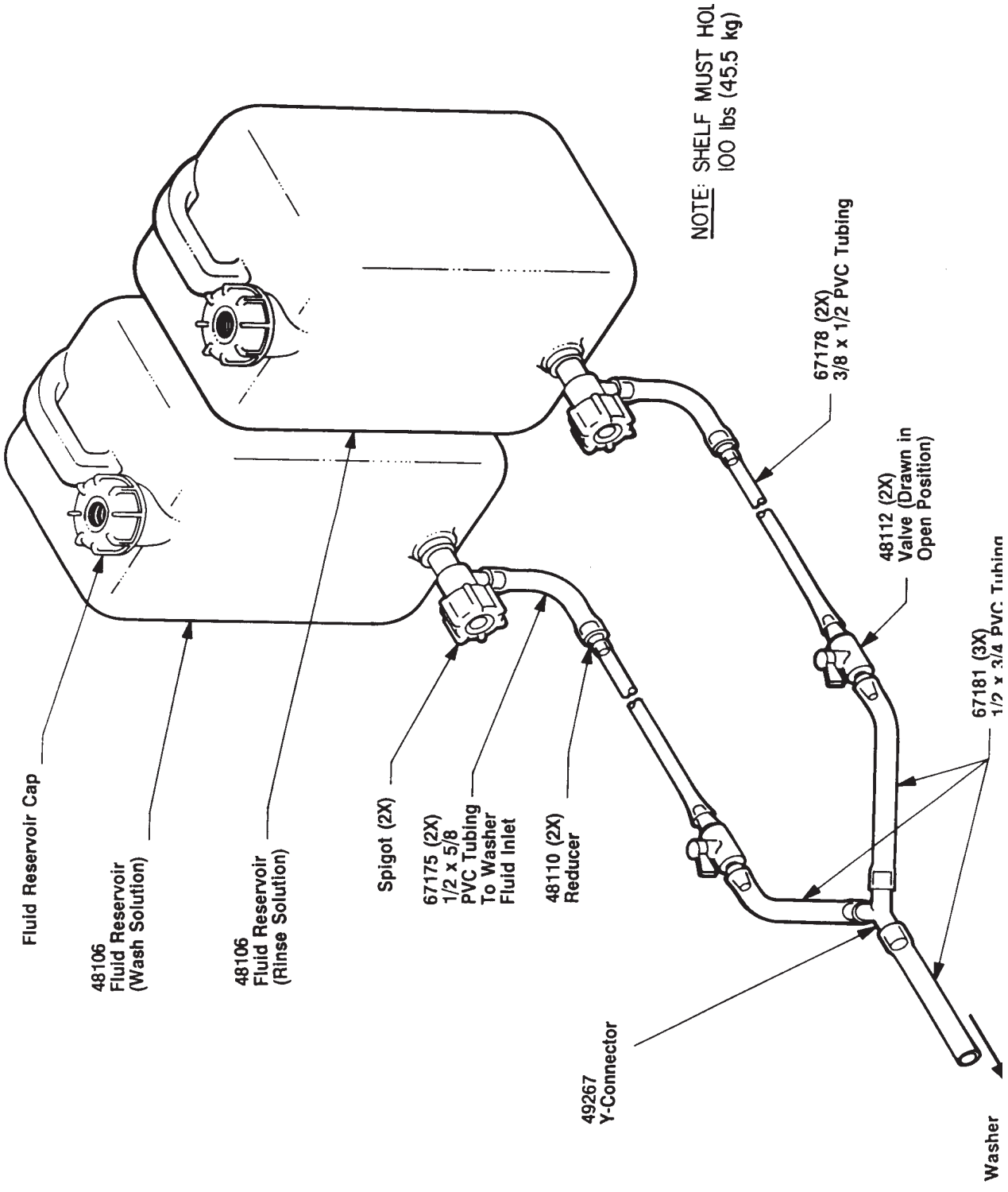
3. **Attach the spigots to the reservoirs and close them.**

4. **Fill the bottles.**

   The 5-gallon (20 liter) bottles are heavy when filled. Decide whether it is best to fill them and raise them, or partially fill them, raise them, and then fill them completely.

5. **Raise the reservoirs to a shelf height of between 26" and 167 " above the working surface of the washer (24 and 165 inches above the washer inlet port).**

   The optimum height for the most precise volume dispensing is 44" above the working surface.


NOTE: SHELF MUST HOL
100 lbs (45.5 kg)
Fluid Reservoir Cap
48106
Fluid Reservoir
(Wash Solution)
48106
Fluid Reservoir
(Rinse Solution)
Spigot (2X)
67175 (2X)
1/2 x 5/8
PVC Tubing
To Washer
Fluid Inlet
48110 (2X)
Reducer
67178 (2X)
3/8 x 1/2 PVC Tubing
48112 (2X)
Valve (Drawn in
Open Position)
67181 (3X)
1/2 x 3/4 PVC Tubing
49267
Y-Connector
Washer

6. **Connect the tubing to the spigots and the inlet port of the washer.**

7. **Once connected, cut any excess tubing to minimize twists, turns, and kinks in the tubing which can hinder flow.**

8. **Make sure the exit port of the bottle is pointed downward so that the tubing does not kink.**

9. **Once set, make sure the inlet port of the bottle is open to air (cap just resting on top).**

10. **The appropriate spigot and valve should be open when operating the unit.**

Optimizing the Setup

- When you are satisfied with the basic setup, cut the tubing if you are not using its full length.

- If the bottles and washer are not at the same height, add 0.40 psi for every 12" that the bottles are positioned below the washer's inlet port; subtract 0.40 psi for every 12" above.

Priming the Tubing for Gravity Feed

1. **Connect the tubing to the inlet port of the washer.**

2. **Completely open the spigot and the lower valve for the fluid to be used to allow the fluid to reach the inlet port of the washer.**

3. **Make sure that the reservoir is open to air.**

4. **When the descending fluid has filled the tubing up to the valve, close the valve and gently manipulate the tubing to allow most of the bubbles to proceed up the tubing into the reservoir. Close the valve until ready to use.**

CHAPTER 3

Operation

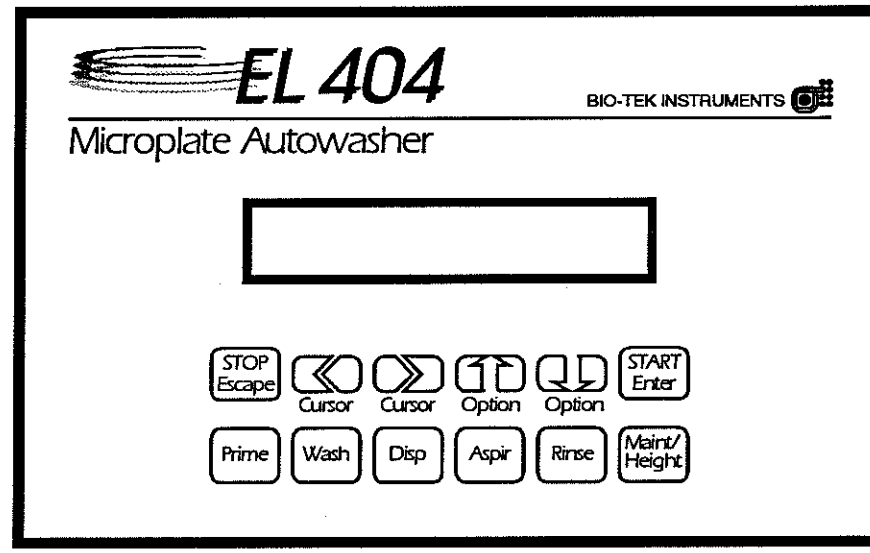
This chapter provides comprehensive information on the instrument. It explains in detail —

- The layout of the EL404 keyboard and the function of each key
- Selecting and loading the microplate
- Creating and Editing a wash program
- Aspiration Depth and Dispense Overflow Height settings
- Priming the system and setting the pressure
- Testing Aspiration depth and Dispense Overflow Height settings

The EL404 Keyboard

The EL404 keyboard allows the user to enter and save values for 10 different wash programs, as well as specify the type of prime, and maintenance routine desired.

When keys are pressed on the EL404 front panel, information, prompts and error messages appear on the two-line, 24-character LCD (liquid crystal display). The illustration below shows the layout of the EL404 keyboard panel overlay.



Each key has a function:.

- Pressing the **STOP/Escape** key halts all operations and returns the instrument to its READY status. All valves close. Pressing **STOP/Escape** again returns carrier to the home (full out) position.

- Press the **START/Enter** key after selecting Prime, Wash, Disp, Aspir, Rinse or Maint/Height keys to see the next prompt on the display, and begin the procedure.

If the function is Wash or Disp, enter the parameters/options for the particular program, then press the **START/Enter** key to execute the selected program.

- Press the **Cursor < >** (right or left) keys to move from parameter to parameter during setup of the wash function.

- Press the **Option** ⇑ and ⇓ keys to move through the options within a given parameter during a wash program or dispense setup. **Option** also allows the user to cycle through the various Prime and Maintenance routines.

- Press the **Prime** key to remove air from the dispense section of the tube manifold and fill that section with fluid to be dispensed.

- Press the **Wash** key to simultaneously aspirate all 96 microplate wells, followed by alternating dispense, soak, and aspirate cycles. The operator can choose from 10 wash programs.

- Press the **Disp** (Dispense) key to dispense fluid into a 96-well microplate. User-programmable wash volumes are available.

- Press the **Aspir** (Aspirate) key to aspirate fluid from all 96 microplate wells.

- Press the **Rinse** key to flush the internal tubing, tube, and valve manifolds with rinse solution. Rinsing consists of emptying the system, priming it with rinse fluid, and then emptying the system. For thorough rinsing of the system, run Overnight Maintenance.

- Press the **Maint/Height** key to select one of four Maintenance programs: Day Maintenance, Overnight Maintenance, Dispense/ Overflow Height and Service Mode..

Powering Up the EL404

Plug in the EL404, power up the unit with the **On/Off** switch located on the washer's rear panel. The washer ejects the carrier, and the display shows:

Bio-Tek Instruments, Inc.
EL404 Version X.XX

X = current version

After a few seconds, the READY prompt is displayed again:

Ready Select a Function

During the Ready state, the EL404 awaits user instruction. The washer has not yet been primed.

Selecting a Microplate

A number of different types of microplates can be used successfully with the EL404. Each time a different brand or type of microplate is inserted, the EL404 must be programmed to ensure the washer operates properly with the new microplate.

Polystyrene microplates.

Round-bottom and some flat-bottom microplates need a round-bottom adapter (shim) to correctly position the aspirate tube in the well. The round-bottom adapter/shim moves the aspirate tube towards the center of the well for maximum evacuation.

PVC microplates

PVC microplates usually require Bio-Tek's optional vinyl plate adapter, although some operate more efficiently without the adapter. Since PVC microplates are flexible, some care should be taken when inserting the plate, and to assure the washer is adjusted for them.

Strip wells

Strip wells must be placed securely in their own carrier frame. All 96 wells must be present. This assembly is placed in the carrier like a polystyrene microplate and requires similar adjustments.

24 and 48 well microplates

If you wish to wash these and other types of microplates not listed here, please call Bio-Tek Product Management.

Loading the Microplate

1. Loosen the screws of the two adjustment brackets on the right and front sides of the carrier.

2. Insert the microplate into the carrier Do not touch the bottom of the microplate as it may leave fingerprints.

- Flat-bottomed microplates: Push the microplate toward the top left corner. Lightly secure the brackets around the microplate, and tighten the screws on the brackets.

  Do not compress the calibration screws in the back and left sides of the carrier by tightening the brackets too tightly around the microplate.

- Round-bottom microplates: Remove the metal shim on the left side of the carrier, and rotate it so that the spacer fits between the carrier and the left edge of the microplate. Tighten the screw on top of the left side of the carrier and push the microplate toward the top left corner. Lightly secure the brackets around the microplate and tighten the screws on the brackets.

3. Manually push the carrier in and see if the dispense and aspirate tubes descend into the appropriate parts of the wells.

- Flat-Bottom microplates: The long aspirate tube should be no more than 1 millimeter from the right side of the well. If it is too close to the side of the well, you may have to insert the shim on the left side of the carrier to push the microplate to the right.

- **Round-bottom microplates:** The long aspirate tube should be closer to the center of the well; usually the shim is required for this.

  The dispense tubes should not be too close to the left side of the well (within 1 millimeter).

- **Polyvinyl plates:** Determine whether a vinyl plate adapter is necessary. Use care in securing the brackets around polyvinyl plates. Refer to the *Optional Accessories* section in this manual, for a list of available stabilizers.

Setting Up Wash Program Parameters

The EL404 stores in memory up to 10 separate washing information files. Each file may be customized by the user to perform a specific set of functions.

- The **Cursor** < and > keys move the cursor backwards and forward from one wash parameter to another, and allow the user to edit the program name.

  As the cursor is moved, the top line of the display explains the selected parameter.

- Option ⇑ and ⇓ keys allow the user to change a parameter from one value to another.

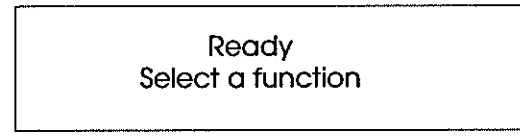
- **START/Enter** key will start the wash program from any wash program option.

- **STOP/Escape** will save the settings, stop the washer at any point, and return the carrier to the home position.

Creating and Editing a Wash program

1. Turn on the washer.

   The EL404 opening screen will appear, and, after a pause, the Ready screen will be displayed.

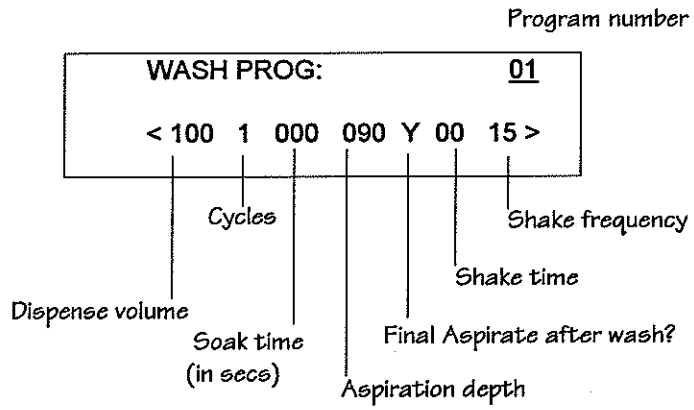


   Place the microplate in the carrier and position the adjustment brackets.

   The shim may be needed if round bottomed wells are beings used. The microplate's corners should be well seated in the carrier.

2. **Press the Wash key.**

   The Wash program display appears, with the cursor positioned under the Wash Program number:



3. **Use the Option ⇑ keys to change the program number.**

   For example, to set up Program 2, use the **Option ⇑** keys until 2 appears on the display next to **WASH PROG**.

   If you plan to use microplates by different manufacturers, set up a program for each microplate, as microwell depths differ.

4. **Use the Cursor ⇒ key to move to the wash volume parameter (refer to the illustration above). Use the Option ⇑ or ⇓ key to incrementally increase or decrease the value.**

   Wash volume: (ul)

   < 100 1 000 090 Y 00 15 >

   The volume increases in 25 microliter increments to 500 ul, and in 100 microliter increments to 999 ul.

5. Use the **Cursor ⇒** key to move to the Number of Cycles parameter. Use the **Option** keys to incrementally increase or decrease the number of cycles.

Number of cycles:
< 100 1 000 090 Y 00 15 >

6. Use the **Cursor ⇒** key to move to the Soak Time parameter. Use the **Option** keys to incrementally increase or decrease the soak time in 5-second intervals.

Soak time (seconds):
< 100 1 000 090 Y 00 15 >

7. Position the cursor under the Aspiration tube depth parameter.

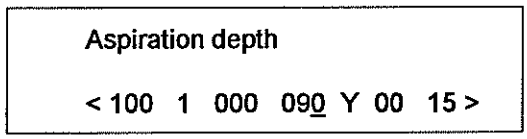
Aspiration depth
< 100 1 000 090 Y 00 15 >

Adjusting the depth of the aspiration tubes in the microplate wells enables appropriate removal of fluid during aspiration.

The EL404 provides 10 user programmable wash programs. Thus, 10 difference aspiration depth settings may be saved to accommodate microplates of varying well depths.

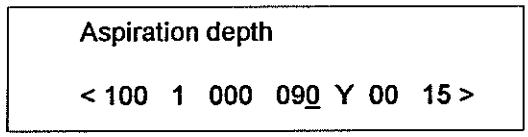
Ensure that the appropriate microplate for this wash program is loaded.

8. Press the **Maint / Height** key once to start the aspiration depth-setting process.



The microplate carrier moves from the home position, and places the aspirate tubes at the last saved depth. If the depth setting is 000, the long aspirate tubes are positioned just above the microplate wells.

9. Adjust the depth with the **Option** keys. Hold down the **Option** key to increment by steps. (Range: 000-200).



Closely monitor the microplate position. When the bottom of the plate nears the tips of the aspirate tubes, release the **Option** key. For most polystyrene flat-bottomed plates, you should release the **Option** key at 090.

10. Press the **Option** key to increment by single steps and look at the tubes entering H1 or H12. The most effective viewing angle for a microplate is at the corner. Stop pressing Option when the aspiration tubes are very close to the well bottoms (a depth of 100-125 for flat bottomed plates).

Do not overdrive the carrier by repeatedly pressing the **Option** key. The carrier has moved too far if it hesitates, stops, and then backs up.

```
Aspiration depth

< 100  1  000  115 Y 00  15 >
```

The aspirate tubes should be near the bottom of the wells at a setting between 100 and 125 for flat-bottomed polystyrene microplates, and between 100 and 130 for strip-well plates.

11. **Press the Option ⇓ key to move the carrier back 4-6 steps from where the aspirate tubes touch the well bottoms.**

```
Aspiration depth

< 100  1  000  110 Y 00  15 >
```

- To leave a residual of more than a few microliters, experiment with the setting.

- It is best to use a buffer solution when adjusting aspiration depth.

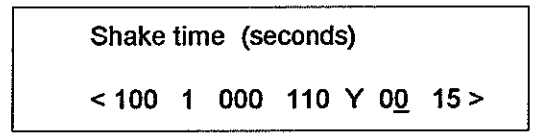
- To leave 4 to 12 microliters in the bottom of a round bottom plate, a setting in the 085 range may be appropriate.

- To leave 50 microliters in the bottom of flat bottom wells, a setting of 090 may be appropriate.

12. Use the **Cursor** ⇔ keys to move to FinalAspirate (after wash). Use the **Option** keys to toggle between:

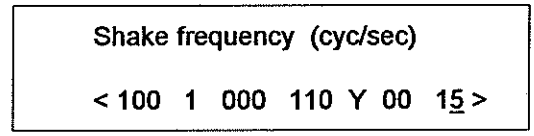
    - Y(es) Aspirate the microplate contents, or
    - N(o) Leave the well contents intact.

13. Use the **Cursor** ⇒ keys to move to Shaking Time. Use the **Option** keys to select from 00 to 30 seconds of shaking.



Selecting 00 means no shaking will occur.

14. Use the **Cursor** ⇒ key to move to Shaking Frequency. Use the **Option** keys to select from 5 to 15 cycles/second.



Twelve to 15 cycles per second is a suggested frequency for ELISA and other immunological assays. Six to 8 cycles per second may be useful for cellular assays. Settings of 9-11 cycles per second may cause excessive noise due to harmonic vibrations and are not recommended. The shaking amplitude is fixed at 2.2 mm.

15. Use the **Cursor** ⇒ key to move to the Program Name parameter.

<OPTION key change name>
WASH PROG 01

Pressing the Cursor keys will return you to the previous screen.

16. If you wish to change the Program Name (i.e. Wash PROG 01 to another name such as Hepatitis), press either **Option** key to move the cursor to the first letter of the program name.

HEPATITIS 01
< 100 1 000 110 Y 00 15 >

Use the Option keys to changes the character from capital letters to small letters, then to numbers and finally symbols.

Edit with CURSOR/OPTION
HEP PROG 04 04

Press **Cursor ⇒** to move to the next space or letter for the name. Up to 20 spaces can be used for the name. That leaves a two character space, and two characters for the program number, which cannot be changed.

17. After editing the name, press the **Cursor** keys to return to the first display, or press **Stop/Escape** to return the instrument to its Ready status.

Dispense / Overflow Height

The dispense / overflow height adjustment sets the height of the aspirate tubes in the wells to prevent fluid overflow during Wash, Dispense and Day and Overnight Maintenance functions.

During aspiration, the carrier moves the microplate the programmed number of steps toward the dispense manifold. After aspiration, the carrier drops to the programmed height for dispensing fluids.

The dispense / overflow height is a global adjustment that applies to all microplates. If both polystyrene and polyvinyl microplates are used, then the dispense / overflow height for the thinner polyvinyl plates may be too high. Using an adapter (Bio-Tek part number 3070068) will raise the polyvinyl plate to the appropriate height.

Setting Dispense Overflow Height

1. Place the microplate in the carrier.

2. Press the **Maint/Height** key.

Day Maintenance
START or OPTION for more

3. Use the **Option** ⇓ key to advance to the Dispense/Overflow Height display.

Dispense/ovrflow height: 008 START or OPTION for more

4. Press **START/Enter** key to start the Dispense/Overflow height routine.

Dispense/ovrflow height: 008 OPTION keys set height

Pressing the **STOP/Escape** key aborts the routine without changing the Dispense/Overflow height.

5. Use the **Option** keys to change the setting.

The **Option** ⇑ key moves the carrier up. The **Option** ⇓ key moves the carrier down. Continuously pressing either a key causes the carrier to move automatically in 1 step increments.

For the optimum dispense / overflow height the aspiration tube should be positioned just inside, or just above the microplate well, where it can siphon dispense overflow.

6. Press either the **START/Enter** or the **STOP/Escape** keys to store the new settings, eject the carrier and return the EL404 display to the Ready state.

Helpful hints:

- During dispensing, the deeper the aspiration tubes are placed in the well, the less fluid remains, and overflow protection is maximized.

- Placing the aspiration tube higher above the well increases the area at the top of the well that is washed. Usually 2-7 steps above the top of the well is the limit before overflow occurs.

Priming the System

The EL404 must be primed before beginning a wash cycle, dispense or Day Maintenance.

Two prime routines are provided:

- Empty Manifold and Prime, and
- Prime Only

The Empty Manifold and Prime routine empties fluid from the dispense chamber and the interior dispense line, then primes the system. This routine is usually used at the beginning of the day to empty the manifold of deionized water, and prime the system with buffer.

The Empty Manifold and Prime routine saves buffer by achieving the appropriate concentration of buffer on the first prime, and eliminating the need for a second prime.

The Prime Only routine primes the system without initially emptying the manifold or interior dispense line.

Both functions draw a large amount of liquid from the washer's dispense bottle, replacing air or whatever fluid is present.

Helpful hints:

- Use Empty Manifold and Prime to replace one fluid with another fluid.
- Use Prime Only to prime an empty system, or to improve on a previous prime. If the Empty Manifold and Prime does not

adequately prime the system, use the Prime Only routine to prime the system again.

- The aspiration depth saved in the last program displayed controls the depth that the aspirate tubes descend in a well during a priming routine.

  For example, Wash Program 1 is set for an aspiration depth of 090 as a default. If Wash Program 1 was the last program displayed, aspiration depth would be set for 090 for priming. If you've customized a Wash Program, and displayed it before priming, be sure the program's aspiration depth setting is at least 070, to prevent flooding.

- To prevent flooding, designate the deepest 96 well plate you have available as a priming plate.

- The clear manifold is provided to inform you how well the system is primed.

- Manifold air bubbles bigger than 1.5" (3.5 cm) in diameter should be removed from the system because they can cause fill volumes to differ. Smaller bubbles are inconsequential.

- If you desire to remove as many bubbles as possible, lift up the front of the washer one inch, and move it from left to right as it primes. This helps the bubbles escape through the diagonal air bleed channel at the top of the manifold.

- To remove a few bubbles without performing the full Prime procedure, press **Prime** and **START/Enter**. When the bubbles have disappeared, press **STOP/Escape**.

Priming a Pressure-Delivery System

The pressure pump provides an adjustable pressure depending on your assay requirements. Higher pressures, such as 3.0 to 5.0 psi, provide for a more vigorous wash. Lower pressures, such as 1.0 to 1.75 psi, may be appropriate for reagents that may not be as strongly bound to the solid phase.

The usable pressure range is from 1.0 psi to 6.0 psi. Lower pressures (around 1.0 psi) cause fluid to drip and may cause the EL404 to register an ERROR in fluid flow. Higher pressures (greater than 6.0 psi) may result in fluid splashing.

The recommended pressure for bottles that are placed lower than the washer is the desired pressure plus 0.4 psi for every one (1) foot (310 cm) the bottles are below the level of the washer.

If very accurate dispensing and minimal usage of priming volume is required, set the gauge for 1.5 to 2.0 psi. Higher pressures than 2.0 cause the fluid to be dispensed to exceed the specified wash volume by small amounts.

As an example, if 250 microliters is requested and the pressure is set at 1.5 psi, 250 microliters will be dispensed. At 3.0 psi, approximately 295 microliters is dispensed. If removal of the excess is desired, then proper positioning of the aspirate tubes will remove the fluid. This means of exceeding the specified volume is highly reproducible: if the washer is set for 250 microliters and 3.0 psi, then approximately 295 microliters will be dispensed every time.

Setting the pressure

1. Make sure all tubing connections and bottle caps are tight.

2. Turn on the pump and observe where the pressure stabilizes.

3. To change the pressure, pull out the locking ring and turn the adjusting knob in small amounts, waiting for a few seconds after each fraction of a turn for the pressure to react, and then stop.

4. To set it at a given pressure such as 1.5, set pressure slightly higher and wait for the needle to settle.

5. Push in the ring when the pressure is satisfactory. You may have to turn the locking ring to the right to lock in the setting.

Empty Manifold and Prime

Use the Empty Manifold and Prime program to replace the volume in a system already primed with a rinse solution such as deionized water. Use Prime Only to prime an empty system. Use Overnight Maintenance to replace wash buffer with deionized water.

1. Ensure that the dispense bottles are filled with the appropriate liquid(s).

   Usually one bottle contains rinse fluid such as deionized water, and the other contains wash fluid such as PBS with Tween.

2. Turn on the pumps and wait for the pressure to rise to the desired level.

3. Open the valve to the appropriate fluid.

4. Press **Prime.**

Empty manifold and prime
START or OPTION for more

5. Press **START/Enter** to begin the Empty Manifold and Prime routine.

Use Buffer Insert plate
Press START/ENT

6. Switch the valve to wash buffer and insert the priming micro-plate into the carrier.

7. Press **START/Enter.** This begins a 90 second routine which empties the manifold, removes fluid from the internal fluid line, then primes the system.

Busy emptying and priming
STOP/ESC to escape

Prime Only

Use this program to prime an empty system or improve on a previous prime. This is also an all-purpose routine for any flushing of the system to clean or decontaminate (see Maintenance).

Prime Only routine

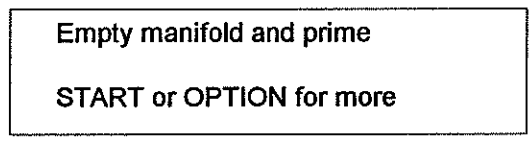
1. Ensure that the dispense bottles are filled with the appropriate liquid(s).

   Usually one bottle contains rinse fluid such as deionized water, and the other contains wash fluid such as PBS with Tween.

2. Turn on the pumps and wait for the pressure to rise to the desired level.

3. Open the valve to the appropriate fluid.

4. Press the **Prime** key.



5. Press either **Option** key to advance to the Prime routine.

Prime
START or OPTION for more

6. Press **START/Enter** to begin the Prime routine.

Use buffer Insert plate
Press START/ENT

7. Switch the valve to wash buffer and insert the priming micro-plate into the carrier.

8. Press **START/Enter.** This begins a 25 second routine which primes the system.

Busy priming
STOP/ESC to escape

If an error message appears on the display during this routine, refer to Chapter 6, *Resolving Problems and Support.*

Gravity-Feed Priming

Priming with gravity feed is very similar to priming with the pressure delivery system with the following additional considerations:

Fluid Reservoir Height

The fluid reservoirs can be placed anywhere from 26 inches (65 cm) to 167 inches (417 cm) above the working surface of the EL404. This measurement is between the level of the bench (that the EL404 is placed upon) and the level of the shelf (that the fluid reservoirs are placed upon).

For the most accurate volumes, the fluid level in the reservoir should be between 44-46 inches above the input port of the washer. Lower fluid levels will dispense lower volumes than that specified.

As an example, if 250 microliters is selected in the wash program, approximately 225 will be dispensed. This volume is very repeatable, however. If dispensing 250 microliters from a height of 26 inches is desired, 275 should be specified.

Gravity Feed Priming

1. Close all valves.

2. Ensure that the top cap of the reservoirs is vented.

   The reservoirs must remain vented during washer operation.

3. Fill the reservoirs with the appropriate liquid(s).

   Customarily, one reservoir contains wash fluid such as PBS with Tween, and the other reservoir contains rinse fluid such as deionized or distilled water.

4. Open the spigot to the desired fluid until the spigot ceases to turn. Then open the appropriate valve(s) in the fluid line.

5. Hand prime the tubing by squeezing the tubing.

   Try to have as direct a line as possible from the reservoir to the back of the EL404.

   It isn't necessary to remove all the bubbles. Only those bubbles larger than 1 inch (2.5 cm) should be removed as they can affect fill volumes.

6. Press **Prime.**

Empty manifold and prime
START or OPTION for more

7. Press either **Option** key to access the Prime routine.

Prime
START or OPTION for more

8. Press **START/Enter** to begin the Prime routine.

Use buffer Insert plate
Press START/ENT

9. Switch the valve to wash buffer and insert the priming microplate into the carrier.

10. Press **START/Enter.** This begins a 25-second routine which primes the system.

If an error message appears on the display during this routine, refer to Chapter 6, *Resolving Problems and Support.*

Testing Aspiration Depth and Dispense Height

To find out if the microplate height adjustment is appropriate, prime the system with deionized water or buffer, run the wash program with a microplate in place, and perform the following steps:

1. Turn on the washer and the pumps.

2. Check the pressure.

3. Insert the appropriate microplate into the carrier.

4. If the washer is not already primed, prime the system with either deionized water or wash buffer. For best results, use wash buffer.

5. If you wish to check the residuals with a balance (scale) zero the microplate after a thorough blotting, or use a new one.

6. Press the **Wash** key.

   Ensure that the microplate is appropriate for the Wash program.

7. Press **START/Enter** twice.

Watch the dispensing fluid to ensure that the dispense/overflow height of the aspirate tubes is what you desire.

8. **Observe the aspiration of the fluid from the microplate.**

   When the carrier finishes moving the microplate up to the aspiration tubes; the motor should not grind.

➲ **Although no harm is done if the aspiration tubes hit the bottom of the microplate, this does not provide optimum aspiration.**

9. **If the motor grinds, reduce the aspiration depth by 20 to 30 steps, and try again.**

   Very few plates require a higher setting than 120 to 130.

10. **When the microplate is brought out, remove it and check the residuals.**

- If too much residual is left, increase the aspiration depth 5 steps and try again. Make sure the aspiration tubes are not hitting the well bottoms.

- If too little residual is left, decrease the aspiration depth.

- If you are using flat-bottomed plates, ensure that the aspiration tubes are not too close to the center of the well. The aspiration tubes should be about 1 to 1.5 mm from the right side of the well.

Operation Checklist for Washing Microplates

As an easy aid to operation of Bio-Tek's microplate washers, the following checklist summarizes the routine operation of the EL404 washer.

- ☐ The washer should be set up with the bottles in their appropriate places.
- ☐ Empty the waste bottles if they are too full.
- ☐ Check the level of wash and rinse fluid in the bottles. There should be at least 2.5 liters of rinse fluid in the rinse bottle.
- ☐ If you are using a pressurized system, make sure the bottle caps are tight.
- ☐ If using a gravity feed system, make sure the bottle is vented at the top.
- ☐ Ensure that the tubing is properly connected and clamped, and that there are no kinks or obvious clogs.
- ☐ Turn on the washer and the pumps.
- ☐ Check the pressure:
  - 1.0 to 1.75 psi for gentle washing of cells
  - 1.5 to 2.0 for most accurate dispensing
  - 3.0 to 5.0 for more vigorous washing

- If the wash and rinse bottles are on the floor, raise the pressure 0.4 psi for each foot below the working surface.

- If you have a vacuum gauge, check the setting. It should be between 10 to 20 inches of Hg.

- Insert the appropriate microplate, i.e., the one used in the last wash, or the deepest microplate available.

- If priming is necessary, use the **Empty and Prime** program to purge the system of one fluid before switching to another (i.e. purging deionized water in preparation for wash buffer), or use the Prime program to prime an empty system.

- If quality control is necessary, perform the dispensing and aspiration checks.

- Press **Wash** to ensure that the settings are appropriate. Take special note of the aspiration depth setting.

- Press **START/Enter.**

- Switch the valve to buffer and close the valve to the rinse solution. Insert the microplate if you haven't done this already.

- Press **START/Enter.**

- When the wash program has completed its cycle, remove the microplate and check for residuals. Make any necessary adjustments.

- Blot the microplate if you wish to remove all residuals.

- Wash more microplates if desired.

- When washing tasks have been completed, perform Day Maintenance with wash fluid, and either leave the washer in this wetted condition, or power down the instrument.

- If additional microplates need to be washed during the day, press **STOP/Escape** to eject the carrier, or simply turn on the washer. When done, perform Day Maintenance with wash fluid.

- At the end of the day, perform Overnight Maintenance and ensure that at least 2.5 liters of deionized water are present in the Rinse bottle.

CHAPTER 4

Functions

Dispense

Aspirate & Rinse

This chapter takes a focused view of the EL404's major functions: Dispensing, Aspiration and Rinsing. It explains —

- How to dispense fluid
- How to aspirate fluid from microplate wells, and
- Rinsing the microplate

About the Dispense Function

The Dispense function initially aspirates the contents of the wells, according to the aspiration depth setting in memory, then delivers a specified volume of liquid to the microplate wells and then returns the microplate to the starting position.

The Dispense function can be used in a variety of ways:

- To ensure that the washer is delivering the proper fill volumes and to determine if any wells are not filling.

- Mass dispensing of reagent. The EL404 dispenses accurate volumes. The specification is ±5% at 350 microliters.

  The EL404 has been used to coat microplates. This often requires dedicating the EL404 for this purpose because of the large dead volume in the product. Switching reagents requires rinsing and repriming.

- Unusual wash cycles. Some multi-plate procedures require very long soak times. These procedures might be more effectively accomplished by inserting the microplate, aspirating and dispensing, and then removing the filled microplate and letting it soak while the next microplate is being aspirated and filled. When all the microplates have been aspirated, filled, and then soaked, they can be inserted in order for the next wash.

How to Dispense Fluid

1. **Ensure that the EL404 is adequately primed before dispensing any fluid into the microplate.**

   The only bubbles that need to be removed are the large ones (more than 1.5" or 3.7 cm in diameter).

2. **Insert the appropriate microplate.**

   The EL404's Dispense program will use as a default the aspiration depth setting in the last wash program displayed. Therefore a microplate similar to the one used in the last Wash program is the appropriate one to use.

   If you wish to use another microplate, select the correct wash program for that plate, or set up a new wash program with an appropriate aspiration depth. To select a wash program, press the **Wash** key, display the correct program, and press the **STOP/Escape** key.

3. **Press the Disp key.**

Dispense volume: 300 ul
OPTION to change START

4. **To change the volume, press the Option keys. The range is 100 to 999 ul/well.**

5. **Press START/Enter to dispense fluid.**

About Aspiration

The Aspiration function removes the contents of the microplate wells and returns the evacuated microplate to the starting position.

This function has two purposes:

- To check for complete aspiration of fluid from the microwells.

- To remove wash solution from soaking wells. It is often necessary to wash microplates and leave them soaking to minimize exposure of the emptied microplate to air. When ready to pipette the next reagent, reinsert the filled microplate into the carrier and press **Aspir** to remove the wash fluid.

How to Aspirate Fluid

1. Insert the filled microplate.

The EL404's Aspiration program uses as a default the aspiration depth setting of the last Wash program used. An appropriate microplate, therefore, would be the one used during the last wash.

- If the aspiration depth setting residing in memory is incorrect for the microplate you are using, select a new wash program, or change the aspiration depth setting.

- To change the aspiration depth setting, display the desired setting and press **STOP/Escape**. It is not necessary to run a wash program.

2. Press the Aspir key, and insert the microplate.

Insert microplate
Press START/ENT

4. Press START/Enter.

Busy
Aspirating

The washer will aspirate the contents of the well, and return the plate to the starting position..

The Rinse Function

The Rinse function empties the manifold, primes the system, and empties the manifold a second time.

The Rinse function has two purposes:

- To switch from one fluid to another: Because Rinse empties and fills the manifold twice, it is used in many cleaning and decontamination procedures.

- To empty the system: After rinsing, pull off the dispense tubing, and press **Rinse**. This empties the valve manifold.

Rinse will not adequately clean the system of wash solution for shutdown. Use Overnight Maintenance for thorough flushing of the system.

Rinsing

1. **Close the valve to the fluid reservoir containing wash fluid (or turn the three-way valve in the appropriate direction).**

2. **Open the valve to the fluid reservoir containing deionized water.**

3. Insert the deepest available microplate.

The microplate selected is usually the one used for priming or maintenance. The aspiration depth used will be that of the last wash program displayed.

Ensure that the aspiration depth in memory is appropriate to the microplate by displaying the wash program, and checking the depth setting. If the setting is incorrect, use another wash program or set up a new one. To store an aspiration depth in memory, display the Wash program and press the **STOP/ Escape** key.

4. Press the Rinse key. Insert a microplate.

Insert microplate
Press START/ENT

5. Press START/Enter.

Busy rinsing
STOP/ESC to escape

To completely empty the manifold, raise the front of the unit by two inches (5 cm), as the manifold empties. After the manifold has emptied for the first time, lower the front of the washer to its original level.

6. **When finished rinsing, the carrier remains in place under the manifold until Prime is pressed.**

7. **To eject or remove the microplate, press STOP/Escape.**

CHAPTER 5

Maintenance

This chapter describes the regular maintenance that should be performed on the EL404, including —

- Daily maintenance (between washes); Overnight maintenance (overnight), and long-term shutdown (for periods longer than 2 1/2 days)
- Quality Control maintenance
- Decontamination of the instrument
- Fuse replacement
- Storage and shipment

Maintenance

Daily, Overnight and Quality Control maintenance should be routinely performed on the EL404 to ensure trouble-free operation. During normal operation, salt crystals can build up and clog the values and tubes. Regular maintenance reduces this problem, eliminates down time, and extends the life of the washer.

Daily Maintenance

Day Maintenance is performed with wash solution on the day that microplates are being washed. This procedure prevents the aspiration and dispense tubes from clogging between washes.

Between microplate washings, Day Maintenance fills a spare microplate with wash solution. Both the dispense and aspiration tubes should extend into the wash solution contained in the wells of the spare microplate.

Day Maintenance

1. **Insert the microplate used for priming or during Overnight Maintenance.**

   Customarily, the deepest microplate or same type of plate used in the last wash is used to prevent the long aspirate tubes from hitting the bottom of the microplate.

2. **Press the Maint/Height key.**

Day Maintenance
START or OPTION for more

3. **Press the START/Enter key.**

Washer primed?
If yes, press START/ENT

   Wash fluid is appropriate to use if the system is already primed with wash fluid.

4. **Press START/ENT.**

   This is the last chance to insert the microplate.

Insert Microplate
Press START/ENT

5. **Press START/ENT.**

Busy dispensing fluid

The washer will dispense about 300-450 microliters, depending on the pressure. It will then immerse the aspirate tubes in the fluid.

After immersion in fluid, the following message appears on the display.

Tubes immersed in fluid STOP/ESC to escape

6. **The washer may be left in this condition until ready to use, or powered off.**

   At the end of the day, you must run Overnight Maintenance with deionized or distilled water.

7. **To return the microplate to the home position, press the STOP/ESC key or turn the washer on.**

If the level of the microplate fluid decreases because of evaporation, run Day Maintenance again.

Overnight Maintenance

Use Overnight Maintenance when the EL404 must be shut down for an extended period of time (such as overnight or for a week-end). Performing Overnight Maintenance on a regular basis helps prevent the formation of salt crystals which can clog tubes, valves and channels.

Overnight Maintenance consists of one rinse and five primes to remove the remains of wash (buffer) solution from the instrument. Two to three liters of rinse solution are used, depending on the pressure.

At the conclusion of Overnight Maintenance, the washer remains in a wetted condition with deionized water, and the display reminds the user to power down the instrument.

Overnight Maintenance

1. **Fill the rinse bottle with 2.5 to 3 liters of deionized water.**

2. **Close the valves to the buffer and open the valve to the rinse solution (deionized water).**

3. **Turn on the pumps.**

4. **Insert the microplate used in priming, Day Maintenance, or the type used in the last wash.**

   Using a deep plate is important in preventing the long aspirate tubes from hitting the bottom of the microplate.

5. **Press MAINT/Height.**

Day Maintenance
START or OPTION for more

6. **Press OPTION ⇑.**

Overnight Maintenance
START or OPTION for more

7. **Press START/Enter.**

   Open the valve to the deionized water and the close all valves to other solutions.

Switch to deionized H2O
Press START/ENT

8. **Insert a microplate. Press START/Enter.**

Insert microplate
Press START/ENT

9. **Press START/ENT.**

The washer runs the rinse program.

Busy rinsing
STOP/ESC to escape

Following the rinse, the washer primes five times.

Busy priming
STOP/ESC to escape

After the primes, the dispense and aspirate tubes are immersed in the deionized water.

10. **The washer beeps three times to remind the user to switch to buffer before washing assay plates.**

If more washing, switch
to buffer Press START

11. **Press START/Enter.**

Tubes immersed in fluid
Turn off or leave powered

The unit may be left on, or powered off at this point. The washer must stay in this wetted condition until it is next used.

12. **Close all valves and spigots.**

13. **To perform another procedure, turn the washer on, or press the STOP/ESC key to return the microplate to the home position.**

    To prime the instrument with wash buffer at this point, use the Empty and Prime routine to purge the system of rinse solution and prime it with wash buffer.

Quality Control Testing

If you are concerned about testing the washer to ensure the wells are being filled, and that all the wells are being properly aspirated, perform the following Quality Control routine:

Dispense Testing the EL404

1. **Insert the microplate into the carrier.**

Ready
Select a function

2. **Press Wash and then the Option ⇑ keys to select the correct wash program.**

WASH PROG 01
<350 1 000 100 N 00 15>

(typical wash display)

Since the dispense program references the aspiration depth setting of the last wash program displayed, ensure the appropriate wash program is resident in memory.

3. Press STOP/Escape to exit the Wash program.

Ready
Select a function

4. Press Disp. Select the desired dispense volume.

Dispense volume: 300ul
OPTION to change START

To easily distinguish the top of the microplate from the top of the solution, select a volume that is 100 ml below the top of the well, such as 250 for flat-bottomed and 200 for round bottomed polystyrene microplates.

5. Press START/Enter.

Dispense volume: 300ul
OPTION to change START

When the microplate rises, the aspiration tubes should not hit the bottom of the microplate. If a grinding noise is heard, or an error message appears on the display, either the wrong microplate was inserted or the aspiration depth needs to be adjusted in the wash program.

6. **Remove the microplate and check each well to see if it contains the desired amount of fluid. Use the stylus to unblock any clogged tubes.**

   If the volumes are widely uneven, or contain some bubbles, check to see how well the manifold is primed. Additional priming may be necessary if one large bubble (greater than 1.5 inches is diameter) is present.

7. **Repeat the dispensing procedure until you are satisfied.**

Testing EL404 Aspiration

1. **Reinsert the filled microplate (used during dispense testing) and press the Aspir key.**

Insert microplate
Press START/ENT

2. **Press the START/Enter key.**

Busy
Aspirating

3. **When the empty microplate is presented, check to see if the residuals are satisfactory.**

4. **Use the stylus to unplug any clogged tubes, and repeat by dispensing more liquid and aspirating again.**

   - If the residuals are unsatisfactory, ensure that the correct wash program is resident in the instrument's memory.

   - If the wash program is correct, check the aspiration depth setting. If the aspiration tubes touch the well bottoms (sound plugged), or you hear a grinding sound, or see an Error message on the display, then the aspiration depth is set too deep for the microplate.

   - The aspiration tubes should be set no more than 1.5 mm from the right side of a flat-bottom polystyrene well.

5. **Repeat the dispense and aspiration procedures as desired.**

Alternative Dispense/Aspiration Procedure Using Wash

If you wish to test the EL404 with a variety of microplates, use this procedure:

1. **Press Wash and select the appropriate wash program containing the appropriate aspiration depth for the microplate you are using.**

   ```
   WASH PROG              01
   <350  1  000  100  n  00  15>
   ```

   If you need to set up a wash program, use the following settings:

- **Volume:** 100 to 250 microliters, depending on the microplate (usually 50-100 microliters below the maximum volume of the well.
- **Cycles:** 1
- **Soak Time:** 000
- **Aspiration depth:** As set for the microplate
- **Aspiration last step:** N (No) and leaving the microplate filled with fluid.
- **Shaking time:** 00
- **Shake frequency:** 15.

2. **Press START/Enter. Insert the microplate.**

Use buffer, insert plate
Press START/ENT to wash

3. **Press START/Enter.**

Busy Washing
STOP/ESC to escape

If the aspiration tubes touch the well bottoms (sound plugged), or an Error appears on the display, then the aspiration depth in incorrect for the microplate, or the microplate is inappropriate for the wash program.

When the filled microplate is presented, remove the microplate and check each well to see if it contains the desired amount of fluid. Use the stylus to unclog any blocked or partially blocked tubes.

If the volumes are uneven, or contain some bubbles, check to see how well the manifold is primed. Additional priming may be necessary if a large bubble (greater than 1.5 inches in diameter) is present.

7. **Repeat the procedure until satisfied.**

8. **Reinsert the filled microplate and press Wash.**

   Change the fifth parameter, Aspirate after last dispense, from **N** to **Y** to bring the plate out empty.

WASH PROG 01
<350 1 000 100 n 00 15>

9. **Press START/Enter.**

Use buffer, insert plate
Press START/ENT to wash

10. **When the empty microplate is presented, check the residuals to see if they are as desired. Use the stylus to unplug any clogged tubes, and repeat by dispensing more fluid and aspirating again.**

If the residuals are unsatisfactory, ensure that:

- The correct wash program is resident
- The aspiration depth setting is correct. If the aspiration tubes sound plugged, a grinding sound occurs, or an error message appears on the display, the aspiration depth setting is too deep.
- The aspiration tubes should be set no more than 1.5 mm from the right side of a flat-bottomed polystyrene well.

Adjusting the Wash Program after Quality Control Testing

If you had to change the settings on a wash program in order to test the EL404, you should return the parameters for *volume*, *number of cycles*, *soak*, and *aspirate last step* to the original settings.

If you had to change the aspirate depth more than 10 units from the original wash program setting, then the wrong program was used, the wrong microplate was used, or the instrument malfunctioned. If you believe a malfunction has occurred, contact Bio-Tek's Technical Assistance Center.

Long-Term Shutdown

When the washer is shut down for an extended time (longer than a weekend),

- The washer should be kept in a wetted condition by running Day Maintenance every day, or
- The washer should be flushed with 0.01 Normal NaOH and 0.01 Normal HCl solutions and left empty. Thorough rinsing will prevent the formation of salt crystals that can clog tubes and channels.

Bio-Tek recommends flushing the washer and leaving it empty since it is difficult to maintain the washer in a constantly wetted condition

Constant Wetted Condition

1. **Turn on the washer, pump(s), and open the valve to the rinse solution such as deionized or distilled water. Make sure that any valves to the wash solution are closed.**

2. **Insert the appropriate microplate for the last wash program used, or select the deepest microplate available.**

3. **If the system needs priming, prime it now.**

4. **Press the Maint/Height key and run Day Maintenance, following the procedure in this chapter and prompts on the washer's display.**

5. **When Day Maintenance has been completed, power off the washer and close all valves.**

6. **Repeat this procedure daily.**

Flushing the EL404 for Long-Term Shutdown

1. **Turn on the washer, pump(s), and open the valve to the deionized water rinse solution. Make sure that any valves to the wash solution are closed.**

2. **Press the Rinse key, insert the same type of microplate used in the last wash, or the deepest available microplate, and press START/Enter.**

3. **Prepare a 1.0 liter solution of 0.01 Normal NaOH.**

   - Molecular weight of NaOH is 40.01 grams/mole.

- 0.01 moles/liter x 1.0 liters = 1.0 x 10^{-2} moles NaOH
- 40.01 grams/mole x 1.0 x 10^{-2} moles = 4 x 10^{-1} grams

- Therefore add 0.4 grams NaOH to 1.5 liters of H_2O.

4. Place the solution into either a separate bottle or empty the rinse bottle and place the NaOH into it.

5. Prepare a 1.0 liter solution of 0.01 Normal HCl.

- Concentrated HCl is 12 Molar (36% to 38%)
- 0.01 moles/liter x 1 liter = 1 x 10^{-2} moles HCl
- 12 moles/liter x "?" liters = 1 x 10^{-2} moles HCl
- "?" liters = 1 x 10^{-2} moles/12 moles per liter
- "?" = 8.333 x 10^{-4} liters
- "?" = 0.833 milliliters of 12M HCl

- Therefore adding 0.833 m*l* of 12M HCl to 1 liter H_2O yields a 0.01 molar solution of HCl.

6. Connect the bottle containing the NaOH solution to the tubing.

7. Select the Prime key, and prime the washer with the NaOH solution.

If you are using a gravity feed system, prime the tubing to eliminate most bubbles.

8. Prime the washer 3 more times.

9. Press the Rinse key to rinse the washer with the NaOH solution and leave the manifold empty. Discard the remaining NaOH solution.

10. Put the 0.01 Normal HCl solution into an empty bottle and connect that bottle to the tubing previously connected to the NaOH solution.

11. Repeat Steps 7, 8, and 9 with the HCl solution.

12. Put 1.5 liters of deionized water into an empty bottle and connect that to the tubing previously connected to the NaOH and HCl solutions.

13. Repeat Steps 7, 8, and 9 with the deionized water.

14. Place a paper towel or another absorbent material under the inlet fitting at the back of the washer.

15. **Close all valves and disconnect the dispense tubing from the inlet fitting.**

16. **Unscrew and remove the inlet nozzle, and clean the in-line filter.**

17. **Elevate the front of the EL404 by 1 to 2 inches.**

18. **Press the Rinse key and insert the microplate.**

    During this cycle, fluid will drain out of the inlet port.

19. **Disconnect the vacuum tubing from the outlet port and use this action to suction any residual fluid from the washer inlet port.**

20. **Put the filter back into the fitting and screw this assembly back onto the inlet.**

21. **Power down the instrument.**

    Occasional dripping from the dispense tubes (the short tubes) is normal. A microplate should be loaded in place to catch any drips after rinsing the washer.

Flushing to Remove Fungi or Proteins

This procedure is used when any kind of fungal growth has occurred anywhere in the system or if proteins have adhered to the tubes resulting in higher background absorbances. This procedure is similar to the one used for long-term shutdown except that the washer is not emptied, and the tubes are cleaned with a stylus.

1. **Turn on the washer, pump(s), and open the valve to the deionized water rinse solution.**

   Make sure that any valve to the wash solution are closed.

2. **Press the Rinse key, insert a microplate of the same type last used for washing, or the deepest microplate available, and press the START/Enter key.**

3. **Prepare a 1.0 liter solution of 0.01 Normal NaOH.**

   - Molecular weight of NaOH is 40.01 grams/mole.
   - 0.01 moles/liter x 1.0 liters = 1.0×10^{-2} moles NaOH
   - 40.01 grams/mole x 1.0 x 10^{-2} moles = 4×10^{-1} grams
   - Therefore add 0.4 grams NaOH to 1.5 liters of H_2O.

4. **Place the solution into either a separate bottle or empty the rinse bottle and place the NaOH into it.**

5. **Prepare a 1.0 liter solution of 0.01 Normal HCl.**

- Concentrated HCl is 12 Molar (36% to 38%)
- 0.01 moles/liter x 1 liter = 1 x 10^{-2} moles HCl
- 12 moles/liter x "?" liters = 1 x 10^{-2} moles HCl
- "?" liters = 1 x 10^{-2} moles/12 moles per liter
- "?" = 8.333 x 10^{-4} liters
- "?" = 0.833 milliliters of 12M HCl

- Therefore adding 0.833 m*l* of 12M HCl to 1 liter H_2O yields a 0.01 molar solution of HCl.

6. Connect the bottle containing the NaOH solution to the tubing.

7. Press the Prime key and use the Prime routine to prime the washer with the NaOH solution.

If you are using a gravity-feed system, prime the tubing to help remove of most of the bubbles.

8. Press the Disp key and set the volume for 100 to 250 microliters, depending on the microplate.

Vinyl microplates should receive 100 microliters, round-bottom polystyrene 200, and flat-bottom polystyrene 250.

9. When the filled microplate is presented, remove and check each well to see if it contains the desired amount of fluid. Use

the stylus to unblock any obstructed, or partially obstructed tubes.

If the volumes are widely uneven or bubbles appear, check to see how well the manifold is primed. You may have to prime again if one bubble (with a diameter greater than 2 inches) or hundreds of smaller bubbles reside in the top of the manifold.

10. **Reinsert the filled microplate, press the Aspir key, and then START/Enter.**

11. **When the microplate is presented, inspect the wells for large amounts of residual fluid. Use the stylus to unblock any obstructed tubes.**

12. **Repeat Steps 8 through 11, as necessary.**

13. **Prime the washer 3 more times.**

14. **Press the Rinse key to rinse the washer with the NaOH solution and leave the manifold empty. Discard any remaining NaOH solution.**

15. **Put the 0.01 Normal HCl solution into an empty bottle and connect the bottle to the tubing previously connected to the NaOH solution.**

16. **Repeat Steps 7, 13, and 14 with the HCl solution for a total of 4 primes and a rinse.**

17. **Put 1.5 liters of deionized water into an empty bottle and connect the bottle to the tubing previously connected to the NaOH and HCL.**

18. **Repeat Steps 7, 13, and 14 with the deionized water, for a total of 4 primes and a rinse.**

19. **Place a paper towel or another absorbent material under the inlet fitting in the back of the washer.**

20. **Close all valves and disconnect the dispense tubing from the inlet fitting.**

21. **Unscrew and remove the inlet nozzle, and clean the in-line filter.**

22. **Put the filter back into the fitting and screw this assembly back onto the inlet.**

23. **Shut OFF all power.**

24. **Occasional dripping from the dispense tubes (the short tubes) is normal. A microplate should be loaded in place to catch any drips after rinsing the washer.**

Decontamination

The following procedure will inhibit the growth of bacteria and fungi in the EL404 channels and tubes. If the washer is to be given to a Bio-Tek representative or sent back to the factory, then before the instrument is packed up, it must be decontaminated if any infectious agents have been used.

Do not use steam autoclaving to disinfect the Model EL404 or its accessories. They can be chemically sterilized as described in the following procedure. We do not recommend using alcohol for this procedure.The new heavy-walled labeled bottles may be autoclaved.

Exposure of anodized aluminum surfaces to bleach solution for more than 30 minutes may cause surface damage. Rinse and thoroughly wipe all surfaces.

Chemical Disinfection

1. **Set up the EL404 fluid inlet and outlet tubes for routine microplate washing. Turn on pumps and open the appropriate valves.**

2. **Pour 1/2 *l* of disinfectant (10% bleach or 0.5% hypochlorite) into the vacuum collection bottle.**

3. **Pour 1.5 *l* of disinfectant (10% bleach) into an empty dispensing reservoir and connect the reservoir to the inlet tubing.**

   If using gravity feed, 2 to 3 liters may be needed depending on the position of the fluid exit port.

4. **If using gravity feed, hand prime the inlet tube by squeezing it.**

   This forces air up into the fluid reservoir and allows the solution to fill the inlet tubing.

5. **Place a microplate into the carrier.**

   Use a microplate similar to the one used in the last wash.

6. **Press the Prime key to prime the EL404 with disinfectant.**

7. **Prime the washer 3 more times.**

8. **Press the Disp key and set the volume for 500 microliters.**

   Make sure that the height adjustment is correctly set for this microplate. During the dispensing cycle, the long aspirate tubes should reside just inside the top of the wells. If they are too high, overflow of bleach will occur; if they are too low, the tubes will be insufficiently immersed.

If you are very certain that the height adjustment is correct, you can set the volume for 999 microliters; if you are not, press the **Maint/Height** key, then press the **Option ⇑** key twice to enter the Dispense/overflow height routine. Check the dispense overflow height.

9. **Press START/Enter to dispense the disinfectant into the microplate.**

   After the microplate rises to aspirate, it will descend to dispense, aspirating any excess that is dispensed. **You want to trap disinfectant in the aspirate as well as the dispense tubes.** The instant that the well fills and the aspirate tubes begin to remove any excess, turn the washer **off** by using the **On/Off** switch on the rear panel of the washer.

   After turning the washer **off,** turn it back **on.**

10. **Inspect the microplate to ensure that all wells are filled. Stylus any tubes that are blocked or partially blocked.**

    Repeat Steps 8, 9 and 10 until stylusing is no longer required.

11. **Shut off the pumps and the washer.**

12. **Reinsert the microplate and push the carrier from the left side of its cross rail into the manifold to immerse the tubes in the disinfectant. When it has stopped under the manifold, pull it**

out 2 to 3 mm to leave room for free fluid circulation into the tubes.

13. **Allow the washer to soak in this condition for 30 minutes.**

14. **After the 30-minute soaking period, turn on the pump(s) and the washer and close the valve to the disinfectant. Open the valve to the deionized water.**

15. **With the microplate in the carrier, press the Aspir key and START/Enter key to remove disinfectant from the microplate.**

16. **Follow the Overnight Maintenance procedure detailed in this chapter. Ensure 2 to 3 liters of deionized water are present in the rinse bottle.**

17. **After completing the Maintenance procedure, press the Rinse key to rinse the washer and to ensure adequate disinfectant removal.**

18. **Turn off the washer and pump(s).**

19. **Shut off the fluid inlet(s).**

Regular Maintenance

Cleaning the Outer Surfaces

A variety of materials comprise the outer surfaces of the EL404:

- The lower case is epoxy-coated welded aluminum;
- The microplate carrier assembly is anodized aluminum, stainless steel, and acetal;
- The top shroud is ABS plastic and the overlays are poly carbonate.

To clean and disinfect the outer surfaces of the instrument:

1. **Unplug the EL404 and remove the microplate carrier, as described in the following section.**

2. **Using a towel wetted with disinfectant, wipe all external surfaces of the washer.**

   Use only mild detergent solutions to clean the surfaces or wipe with a cloth dampened with 10% bleach.

3. **Using a clean towel wetted with deionized water, thoroughly wipe these surfaces to remove residual disinfectant and prevent corrosion from the bleach.**

4. **Allow all disinfected surfaces to air dry for 30 minutes.**

Cleaning the Microplate Carrier

The carrier assembly is easily removed from the guide rails for cleaning and disinfecting:

1. **Remove any microplates from the microplate carrier.**

2. **Remove the microplate carrier by reversing the procedure detailed in Chapter 2, “Installing the EL404” .**

3. **Soak the carrier in a disinfectant such as 10% bleach. The carrier cannot be steam autoclaved.**

Cleaning the Tube Manifold

The tube manifold , which holds the aspiration and dispense tubes, is located underneath the front control panel on the EL404

Ninety six small-diameter dispense tubes and 96 small-diameter aspirate tubes are mounted in the tube manifold, which also contains 12 aspirate channels.

Strict adherence to the preventative maintenance procedures described in this chapter should keep all tubes and channels clean and ensure reliable performance of the EL404. However, since wash solutions can contribute to salt formation, the tube manifold is designed to be disassembled and cleaned by the operator.

To clean the tube manifold:

1. **Turn on the power to the EL404, and remove and clean the microplate carrier.**

2. **Reinstall the microplate carrier, and load a microplate.**

   Be sure that the corners of the microplate have been firmly pressed into the corners of the microplate carrier.

3. **Connect the vacuum tube to the EL404 and turn on the vacuum pump.**

   It is not necessary to connect the dispense tubing and fluid reservoirs during cleaning of the tube manifold.

4. **Elevate the front of the EL404 one or two inches.**

5. **Press the Rinse key to removes any fluid from the tube manifold.**

   If the microplate is not mounted securely in the carrier, a warning tone will sound and the display will alert the user that the microplate has been incorrectly inserted.

6. **At the completion of the Rinse cycle, shut off the vacuum pump.**

7. **Power down and unplug the EL404.**

8. **Remove the microplate from the carrier.**

9. **Use the 3/16" Allen wrench provided with the EL404 to remove the two (2) shoulder screws which connect the tube manifold to the valve manifold.**

There are three (3) O-rings that seal the tube manifold and valve manifold together. They can be removed for cleaning at this point if any debris is noted around their edges.

The O-rings can be washed with a towel dampened with deionized water; they should be thoroughly dry before they are placed between the manifolds.

➲ **Use caution when handling the tube manifold, as tubes can be bent or broken.**

10. **Turn the tube manifold over to expose the protruding dispense and aspirate tubes. Carefully clean any clogged tubes with the stylus that is provided with the EL404.**

11. **If the cleaning is sufficient, the unit can be reassembled by replacing the O-rings and then mounting the tube manifold on the valve manifold by tightening the 2 shoulder screws.**

12. **If more cleaning is required, use a 9/64" Allen wrench to remove the eight (8) screws that hold the two top and bottom pieces of the tube manifold together.**

13. **Hold the manifold in the horizontal (flat) position, and gently pull the manifold apart.**

    There are two additional O-rings that seal the top and bottom of the manifold together. Remove the rings if they need cleaning. Clean the rings with a towel dampened with deionized water. The rings should be dry before they are replaced.

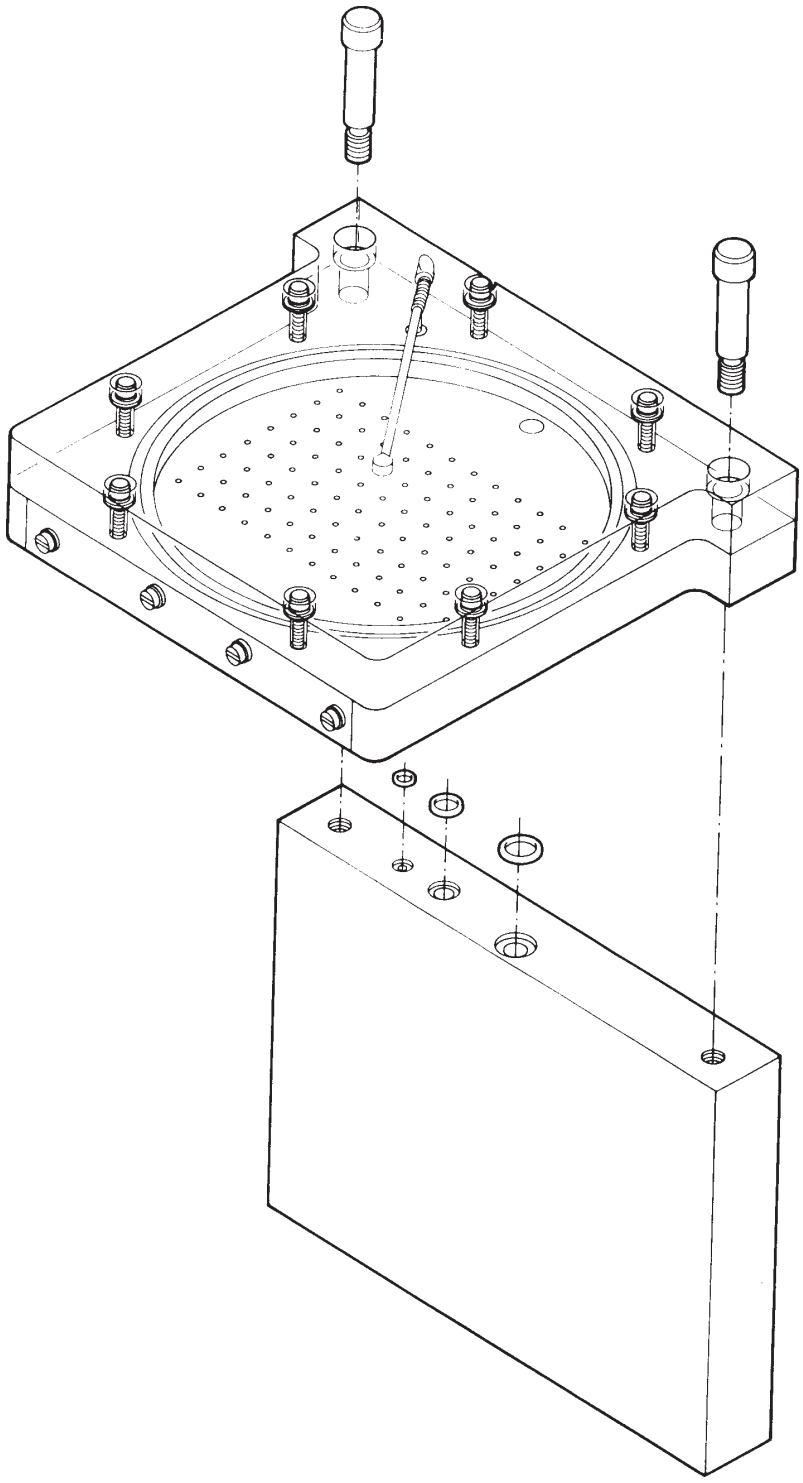


***Figure 6-1**: Tube Manifold to Valve Manifold Assembly*

14. **Remove the stainless steel plate with four (4) screws on the front edge of the tube manifold, using a 3/32" Allen wrench.**

    The plate plugs the ends of the individual aspiration channels.

15. **Soak the plate with the grey polyvinylchloride (PVC) bottom of the manifold in deionized water.**

    The PVC portion of the plate can be placed in a vibrasonic bath (e.g., a sonicator) for 15 minutes to remove any blocks or salt buildup.

16. **Use the stylus to clean any clogged tubes.**

17. **Locate the air bleed outlet on the top half of the tube manifold, and use a syringe, or a forced water source, to clean the air bleed channel.**

18. **Wipe the clear top half of the manifold with a towel dampened with deionized water. Allow to air dry.**

19. **Reassemble the manifold when all parts are dry. Replace the stainless steel plate with the 4 screws that plug the aspiration channels.**

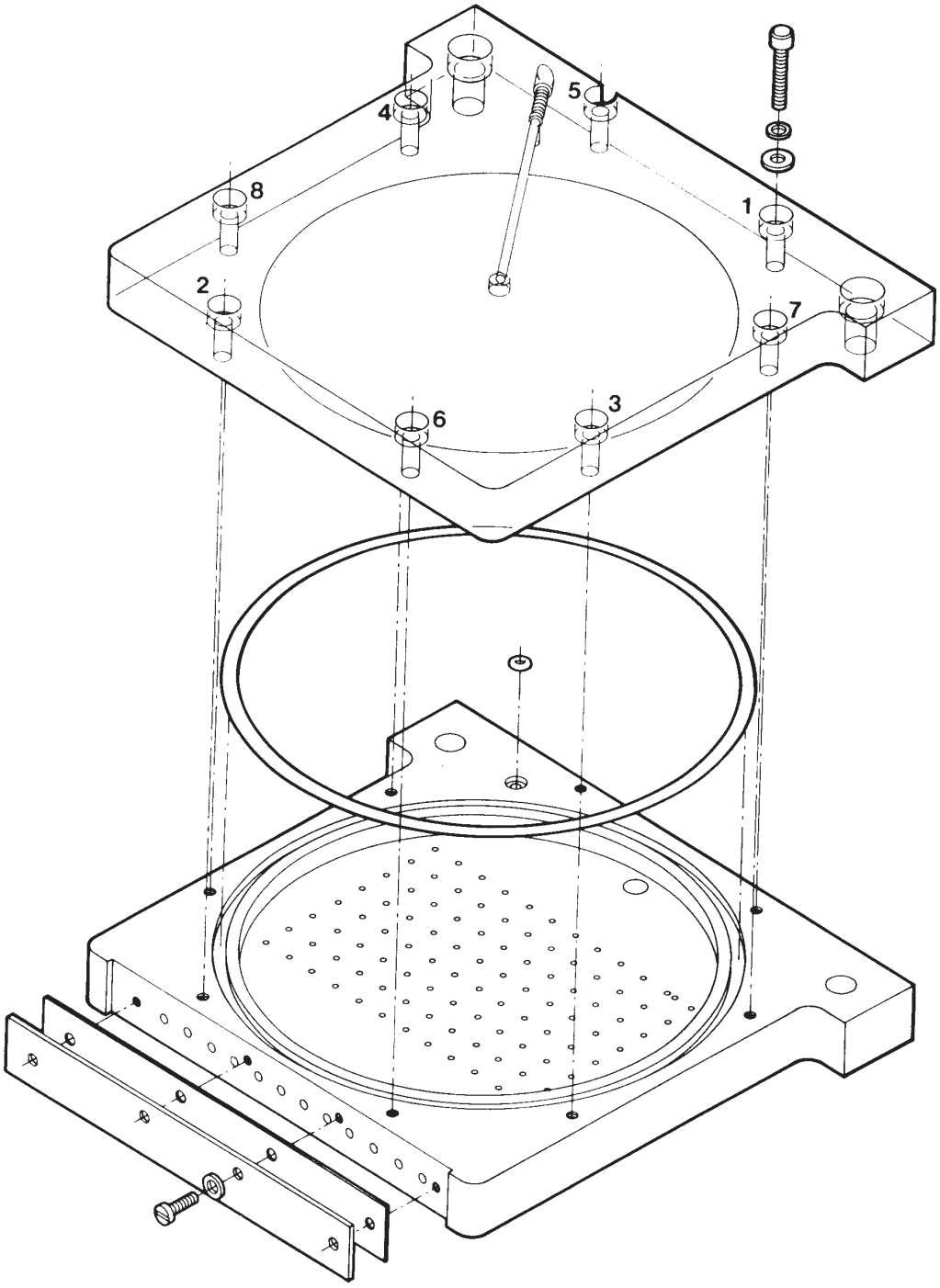


Figure 6-2: *Tube Manifold Assembly*

20. **Replace the two (2) O-rings to seal the two halves of the manifold.**

21. **Place the two (2) shoulder screws into the assembled tube manifold to properly align the manifold.**

22. **Tighten the eight (8) screws that hold the manifold halves together.**

    Replace the screws in the order called out in **Figure 5-2** to ensure that pressure is applied evenly and that a good seal is created. Be sure to insert the two (2) metal washers between the manifold and screws. Do not overtighten the screws, as they are threaded in plastic.

23. **Replace the three (3) O-rings that seal the tube manifold with the valve manifold (see Figure 5-15).**

24. **Position the tube manifold on the valve manifold and carefully tighten the shoulder screws using the Allen wrench.**

    If the O-rings in the manifolds are lost or damaged, they can be ordered in kits from Bio-Tek by using the part numbers that follow.

O-Ring Kits for EL404 Manifold Assembly (Spare Parts Listing)

Use the part numbers that follow to order O-rings, either singly or in kits for the EL404 Valve and Tube manifolds. O-Rings and kits may be ordered by contacting your Customer Service representative, or through Bio-Tek's Technical Assistance Center.

Valve Manifold O-Ring Kit	4020134*
• small O-ring	49259
• medium O-ring	49260
• large O-ring	49261

Tube Manifold O-Ring Kit	4020135*
• small O-ring	49259
• large O-ring	49265

* Order O-ring kit by using this part number.

Replacing Fuses

The EL404 fuse bay is located on the Power Supply module on the back panel of the instrument.

To replace fuses:

1. **Turn off the EL404, and unplug the main power cord.**

2. **Using a small-blade screwdriver or similar tool, pry open the fuse Access cover.**

   The fuse block assembly is on the back side of the cover. Refer to Figure 5-3.

3. **Carefully lift the old fuse from the fuse slot, and replace the fuse with a new one.**

Warning: Using other than the specified fuse can cause fire and/or shock.

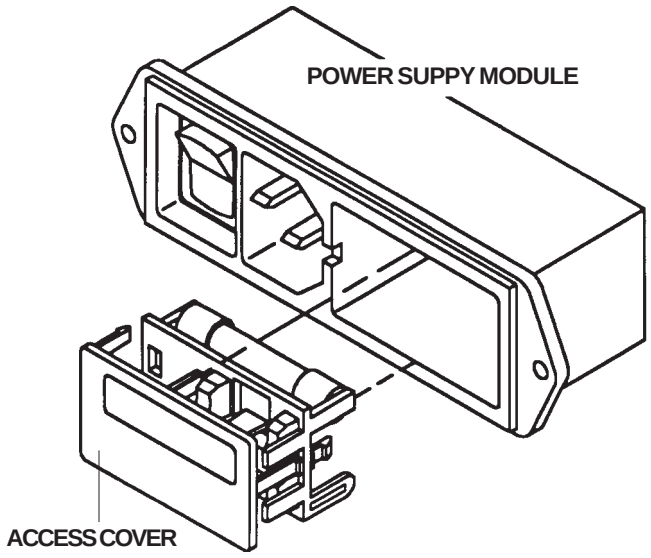


Figure 5-3: *Fuse Assembly*

Configuring the EL404 for Different Voltages

If the EL404 was purchased within the continental United States, it has been factory set for U.S. voltage (either 110 or 120 volts). If you wish to use the washer at a location with 220 or 240 line voltage, the fusing must be reconfigured.

Set for U.S. current, the fuse block is mounted on the backside of the Access cover with the jumper bar exposed, providing one slot for a single fuse (Figure 5-4).

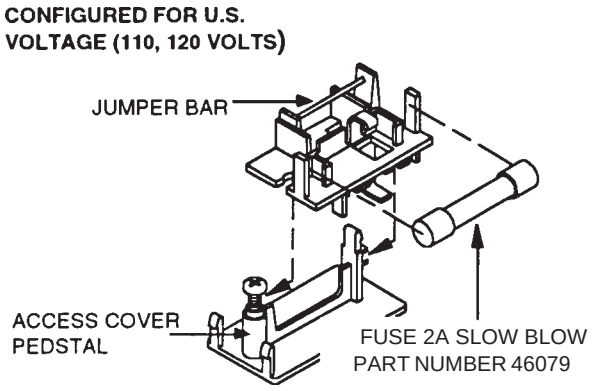


Figure 5-4: *Fuse Configured for U.S. Voltage*

To reconfigure the fusing for International voltages:

1. **Loosen the Phillips screw that attaches the fuse block to the access cover (2 turns).**

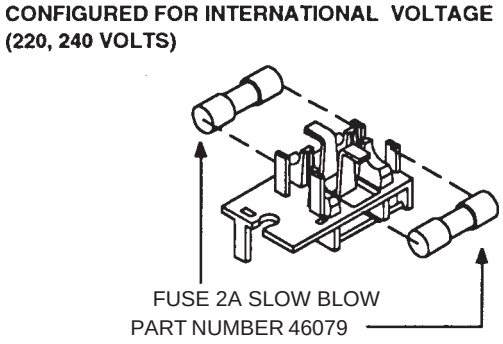


Figure 5-5: *Configuring the fusing for International Voltage*

2. **Slide the fuse block away from the cover. Turn the fuse block over so that the jumper bar lies flat against the cover and the two fuse slots face up (Figure 5-5).**

3. **Slide the fuse block back onto the Phillips screw and pedestal, and tighten the screw.**

   Overtightening the screw may cause stripping of the threads on the fuse holder.

4. **Replace the cover to the fuse assembly, and plug in the EL404.**

Storing the EL404

The EL404 should be stored on a flat surface that is relatively free of vibration. The storage environment should be free of dust and other foreign particles.

The EL404 can be stored under the following environmental conditions:

- temperature: -20° to +50° C (-4° to +122° F)
- humidity: up to 95%
- altitude: 7,620 m (25,000 ft)

The EL404 should be protected from temperature extremes that can cause condensation within the unit and from corrosive fumes and vapors.

Shipping the EL404

If the EL404 is shipped to Bio-Tek for service or repair, the washer **must** be shipped in the original packing. Other forms of commercially available packing are not recommended and can void the warranty.

If the original packing materials have been damaged or lost, contact Bio-Tek for replacement packing.

Before shipping the instrument:

- Obtain a **Return Authorization Number** from Bio-Tek Service, (800)-242-4685. This Return Authorization Number must appear on the outside of the shipping box.
- Include a statement of what is required of the Bio-Tek Service Department (state whether the unit requires calibration, cleaning, or repair).
- Include a tag which includes the Return Authorization Number, the full model number, and the serial number.
- Provide Bio-Tek with the name and telephone number of a person who may be contacted if questions arise.
- Insure the instrument for full value.

Repacking the EL404 for Shipment

Before the EL404 is returned to Bio-Tek for service or repair, the decontamination described in this chapter must be performed.

After decontamination, use the following checklist to repack the instrument:

1. **Remove the microplate from the carrier.**
2. **Remove the microplate carrier from the instrument.**
3. **Remove the aerosol cover, if used.**
4. **Wrap the aerosol cover and the microplate carrier in protective material such as bubble wrap.**
5. **Place accessories (if they are to be returned) in the box with protective foam packing.**
6. **Replace the foam and repack the box, including any envelopes and bags.**
7. **Place the washer in the carton with any other pieces.**
8. **Store the accessories in the side compartments and the carrier in the top foam piece compartments.**
9. **Place the top foam piece into the box on top of the washer.**

10. **Close the box and reseal. Mark the box as Fragile.**

11. **Write the return authorization number on the outside of the box in plain view.**

12. **If the pumps and/or bottles are to be returned, place them in a separate box and protect them suitably.**

CHAPTER 6

Resolving Problems & Support

Every effort has been made to ensure the EL404 is both extremely reliable and easy to use. Nevertheless, you may run into problems. In this chapter you will find helpful information to help resolve any problem you might have. This chapter includes —

- How and when to contact Bio-Tek's Technical Assistance Center
- Troubleshooting the EL404
- Error Messages

Resolving Problems With the EL404

This chapter is designed to help you isolate and solve problems that may occur with the EL404, and provides a recommended procedure.

If the procedure fails to correct the problem, or the instrument needs to be serviced by Bio-Tek, contact Bio-Tek Service (customers inside the USA) or contact your local service representative (customers outside the USA). You may contact Bio-Tek by telephone or by Fax.

Whichever method you choose, please provide the following information:

- EL404 Serial Number and Software version (displayed at powerup);
- The specific steps which reproduce your problem;
- Any error codes displayed on the screen (Descriptions of error codes are available in this chapter)
- A daytime telephone number where you can be contacted.

If you are unable to resolve the problem, contact Bio-Tek for a Return Authorization Number, repack the washer properly, and return the instrument to Bio-Tek for servicing.

Written Communications

If you prefer, you can write a letter with your comments and send it to:

Bio-Tek Instruments, Inc.
Technical Assistance Center
Highland Park, Box 998
Winooski, VT USA 05404-0998

Phone Support

You can telephone the Technical Assistance Center from 8:30 AM to 6 PM EST and/or Customer Service between 7:30 and 5 PM. EST, Monday through Friday, except Holidays. Emergency Technical Assistance is available Monday through Friday from 6-9 PM EST through the TAC answering service. The numbers are:

- **Bio-Tek Instruments (Main Number)**
  (802) 655-4040
- **Technical Assistance Center:**
  (800) 242-4685
- **Customer Service:**
  (800) 451-5172
- **International Customer Service & Service Department** (in U.S.):
  (802)-655-4740
- **International Customer Service & Service Department**
  (Belgium):
  +32-3-829.04.55

Fax Support

You may Fax your questions and /or requests for help 24 hours a day to the following numbers:

- **Technical Assistance Center:**
  (802) 655-3399
- **Customer Service:**
  (802) 655-7941
- **International Customer Service** and **Service Department** (in U.S.):
  (802) 655-3399
  (802) 655-7941

- **International Customer Service** and **Service Department** (Belgium):
  +32-3-825.47.49

Before You Begin Troubleshooting

Use this section as a guide to help you diagnose the cause of simple washer problems. You should contact Bio-Tek's Technical Assistance Center for service instructions if:

- You have attempted the troubleshooting procedures in this chapter, or are experiencing instrument performance problems that you are unable to diagnose.

- A visual inspection of the components, or running the EL404 in "Service Mode" fails to reveal the source of the problem.

- A regular, contracted warranty maintenance is scheduled;

Service Mode

You may wish to check the EL404 washer while it is in "Service Mode." Service Mode is the software routine that allows the user to perform a "dry" check of washer's valve function of the washer. To operate the washer in Service Mode:

1) Push the microplate into position and close all outside valves;

2) Press the Maint/Height key

3) Press the Option key to advance to Service Mode;

4) Press the Start/Enter key.

5) Check the washer valve action by pressing the appropriate Option or Cursor keys.

Start-Up Problems

❖ Display not on:

- Power cord not plugged in

➪ *Check power connection.*

- Fuse blown

➪ *Inspect fuse; replace if necessary*

❖ Vacuum pump won't start, or shakes when turned on:

- Too much residual vacuum force for diaphragm pump.

➪ *Release the vacuum by loosening the tubing from the pump or unscrewing a bottle cap. Reconnect and start again.*

Start-Up Checklist

- ■ Make sure the pump(s) are on.
- ■ Check tubing connections/clamps. Ensure tubing is connected to the vacuum sensing switch.
- ■ Check for kinks; clogged tubes (valves, Y-connectors, inlet and vacuum protection filters).
- ■ Make sure tubing and/or bottle valves are open.
- ■ Ensure wash/rinse bottles contain enough fluid.
- ■ Ensure there is sufficient pressure and vacuum force.

Pressure Delivery System Checklist

- Are there large air pockets in the tubing? Prime system.
- Is pressure at least 1.5 psi (1.5 psi + 0.4 psi for every foot below washer if the bottles are on floor)?
- Are valves open to liquid? Do valves have an inside diameter of 1/4"?
- Are bottle caps/tubing connections tight?
- Do tubing connections have clamps? Any leaks?
- Are gaskets on bottle caps and disconnects leaking?
- Is male part of disconnect clogged or malfunctioning?
- Is the inlet filter clogged? Unscrew the "Dispense In" inlet barb, remove the filter and rinse thoroughly.
- Is tubing kinked or clogged?
- Do you have enough fluid in reservoirs?
- Is the tubing diameter correct? (3/8" inside, 5/8" outside).
- Is liquid flowing freely out of dispense tubing?

✤ IF ALL EFFORTS FAIL:

- Raise pressure to 3.0 or higher; Try a wash or dispense.
- Dispense valve may be clogged or flow transducer faulty. Close the valves and check the dispense valve in Service Mode.

Gravity Feed Checklist

- Are bottle caps open (vented)?
- Is distance between working surface & bottle at least 26"?
- Is tubing inside diameter 3/8" ? Is tubing kinked/clogged?
- Are appropriate valves and spigots open? Does liquid flow freely?
- Is inlet filter clogged? Unscrew the “Dispense In” inlet barb, remove the filter and rinse thoroughly.

✤ IF ALL EFFORTS FAIL:

- Raise fluid level as high as possible ; Try a wash, or dispense
- Dispense valve may be clogged or flow transducer faulty. Close the outside valves and check by running “Service Mode.” Contact Bio-Tek's Technical Assistance Center.

Aspiration Problems

❖ Poor or Uneven Aspiration:

- Not enough vacuum.

➪ *Refer to Aspiration checklist.*

- Clogged aspiration tubes.

➪ *Insert stylus into clogged tubes. If problem reoccurs, clean the tube manifold.*

- Microplate aspiration depth adjustment too high / low.

➪ *Readjust aspiration depth.*

- Microplate not level in carrier.

➪ *Make sure carrier is clean.*

➪ *Reseat microplate in carrier. If problem is unre solved, call Technical Assistance.*

- Strips are not level.

➪ *Level the strips. Use plastic leveler with Immulon strips.*

- Aspiration tubes not horizontally positioned properly in wells

➪ *Use shim or contact Bio-Tek Technical Assistance Center.*

- Top shroud forced behind tube manifold. Movement Rails misaligned.

➪ *Contact Bio-Tek Technical Assistance Center.*

Aspiration Checklist

- Is vacuum pump actually on?
- Is vacuum force between 10" of Hg at 1 ft^3/minute and 20" of Hg at 0.30 ft^3/minute?
- Are all tubing connections secure; bottle caps tight?
- If vacuum tubing is removed from back, is sufficient force exerted with vacuum pump on?
- Is tubing kinked? Collapsing? Tubing needs 1/8" wall, 3/8" internal diameter.
- Are in-line filters (if applicable) clogged?
- Are male/female disconnect parts functioning? Clogged?

Absorbances

❖ Higher Than Anticipated

- Scratches on well bottoms
  - ➪ *Refer to Microplate Scratch Prevention Checklist*
  - ➪ *Use dual wavelength to eliminate optical contribution.*
- Inadequate residual removal
  - ➪ *Refer to Section titled Aspiration Problems*
- Protein and other buildup, especially on aspirate tubes

➪ *Use Flush Procedure to remove fungal growth and protein buildup.Refer to Fluid Dispensing Checklist.*

- Inadequate fluid pressure.

➪ *Some assays require a vigorous wash. Try increasing pressure between 3.0 and 6.0 psi.*

- Wash parameters may need changing.

➪ *Try increasing volume, number of wash cycles, soak time and shaking.*

Scratches

❖ Scratches on Microplate Bottom

- Microplate adjustment set improperly.

➪ *Refer to Microplate Scratch Prevention Checklist*

Microplate Scratch Prevention Checklist

- ■ Aspiration depth correct? Tubes hitting well bottoms?
- ■ Microplate securely positioned in the carrier? Level?
- ■ Strip wells properly placed in their holders? Level?
- ■ Round bottom plates: Use shim stored on the left or front of the carrier. This moves microplate well to the right, centering aspiration tubes over well.

Dispense and Flooding Problems

❖ Uncontrolled dispensing during Wash or Dispense

- Defective valve or flow sensor.

➪ *Close all outside valves and check in Service Mode. Contact Technical Assistance Center.*

➪ *Refer to Fluid Dispensing Checklist*

❖ Unable to dispense

- No microplate in position.

➪ *Insert microplate.*

- Inadequate aspiration height setting and/or plate sensing switch adjustment.

➪ *Adjust, and/or turn white sensing screw two turns to the right. Check to ensure sensing lever is not bent or broken.*

❖ Plate overfills (floods):

- Dispense overflow height too high.

➪ *Reset dispense overflow height adjustment.*

- Aspiration tubes hit bottom of microplate during Prime or Overnight Maintenance.

➪ *Lower aspiration depth setting by 10-20 steps in the wash program currently resident in memory..*

- Vacuum weak.

➪ *See Aspiration Checklist.*

- Quick disconnect on bottle top failed.
- ➪ *Remove main vacuum tube and test for vacuum force.*

- Vacuum valve stuck.
- ➪ *Test while the EL404 is in Service Mode. Call Bio-Tek's Technical Assistance Center.*

❖ Uneven dispensing of fluid; wells not filled

- Clogged dispensing tubes.
- ➪ *Unclog tube with stylus; clean manifold if problem reoccurs;*
- ➪ *Ensure Day and Overnight Maintenance are performed.*

- Manifold or tubing not adequately primed.
- ➪ *Check dispense chamber for bubble greater than 2 inches in diameter; re-prime.*

- Dispense pump pressure too low.
- ➪ *Increase pressure slightly.*

- Dispense valve clogged.
- ➪ *Close all valves; check while in Service Mode;, Contact Bio-Tek Technical Assistance Center.*

Fluid Dispensing Checklist

- ■ Day & Overnight maintenance regularly performed?

- ■ Has unit been flushed recently?

- Large bubble (>2" across) in dispense chamber?

- Air bubbles in tubing after filling bottle(s)?
  To avoid creating air bubbles every time the wash or rinse bottle is filled, make a mark halfway down the bottle and refill when the fluid level has dropped to that point. Unscrew the cap and let it hang over the side just enough to avoid emptying the inside tube and enough to refill the bottle.

- Use stylus on tubes to minimize clogging?
  If you have to stylus many tubes, remove the tube manifold, place upside down on a flat surface, and stylus the tubes.

- Entire manifold disassembled and cleaned in ultrasonic bath?

Fluid Leakages

❖ Fluid leaking from manifold or between tube and valve manifold O-rings

- Defective O-rings.

➪ *Remove manifold and replace O-rings.*

❖ Fluid leaking from the valve manifold

- Defective seals.

➪ *Call Bio-Tek Technical Assistance Center.*

❖ Fluid leaking from the inside of the instrument

- Defective tubing connector or inlet tubing.

➪ *Call Bio-Tek Technical Assistance Center.*

❖ Fluid leaking from external tubing connector

- Defective connector.

➪ *Replace connector.*

- Worn tubing.

➪ *Replace tubing.*

- Worn seal (inlet or vacuum fitting).

➪ *Replace filter or seal.*

❖ Dripping from Dispense or Aspiration tubes

- Pressure too low..

➪ *Raise pressure. See pressure delivery checklist.*

- Defective O-rings in tube manifold.

➪ *Replace O-rings.*

- Defective O-rings between tube and valve manifolds.

➪ *Replace O-rings.*

- Defective valve manifold.

➪ *Return to Bio-Tek for service.*

- Aspiration tubes only: Vacuum low (below 8 in. Hg at 0.67 ft^3)

➪ *Increase vacuum to normal*

Displayed Error Messages

The following error messages may appear on the EL404's display during normal operation. Solutions are given for each message.

❖ No vacuum sensed. Check vacuum and tubing

- No vacuum.

➪ *Refer to Aspiration checklist.*

❖ Insert Microplate. Test screw setting

- Microplate not in position.
- Aspiration depth setting too low.
- The EL404 is unable to sense the microplate.
- Polyvinyl microplate being used.

➪ *Insert microplate.*

➪ *Adjust aspiration depth to value >50.*

➪ *Turn the plate sensing screw two turns to the right to ensure system can sense the microplate. Check to see if the metal microplate sensing switch is bent or broken.*

➪ *Polyvinyl plates may require an adapter or more turns on the microplate sensing screw.*

❖ Inadequate fluid flow. Check pressure and tubing

- Not enough fluid flow.

➪ *Refer to Pressure Delivery and Gravity Feed Checklists.*

❖ Leak in System. Check in Service Mode

- Defective O-rings, seals, connectors inside washer.

➪ *Refer to Fluid Leakage Troubleshooting section.*

- Dispense valve stuck open.

➪ *Enter Service Mode, close all outside valves, and check the dispense valve. See Service Mode instructions in this chapter.*

❖ Carrier not reached home. Check home switch

- Obstruction or faulty home switch.

➪ *Remove obstruction. If problem remains, contact the Technical Assistance Center.*

❖ Check aspiration depth and disp/overflow height

- Dispense/overflow height set to greater number than the number set for aspiration depth.

➪ *Set proper aspiration height in wash program.*

❖ Check plate in carrier. Check aspiration depth

- Tubes collided with top of plate or well bottoms.

➪ *Ensure plate is correctly positioned in the carrier. Decrease the aspiration depth.*

❖ Low Battery

- Battery Failure

➪ *Contact the Technical Assistance center to obtain the correct battery.*

CHAPTER 7

Reference

This chapter provides information on —

- EL404 specifications
- Optional Accessories
- Requirements for User Supplied Accessories

EL404 Specifications

Microplates

The EL404 accommodates all 96-well format polystyrene and polyvinyl microplates: flat, *U* (round), and *V* bottom. A vinyl plate adapter is recommended for use with polyvinyl microplates. Microwell strips must be used in their carrier.)

Speed

The EL404 washes and aspirates 96 wells simultaneously in 7 seconds. One full cycle (13 seconds) includes: movement of carrier into position underneath tube manifold, 1 wash and aspiration cycle, and movement of the carrier back to home position. Each additional cycle adds 7 seconds.

Fill Volume

100 to 500 microliters (25 microliter increments) and from 500 to 999 microliters (100 microliter increments); user programmable.

Wash Cycle

9 wash cycles may be selected.

Soak

0, 5, 10 ... (5 second increments) 0-600 seconds.

Accuracy

At 350 microliters, well-to-well fill variation is $\pm$5%.

Aspiration Efficiency

6 microliters maximum residual/well (average).

Fluid Delivery

Gravity-feed or optional pressure-delivery, transducer-controlled fluid inlet.

Vacuum

Displacement between 0.30 ft^3/min at 20 in Hg and 1 ft^3/min at 10 inHg.

Power Consumption

80 to 250 VAC, 50/60 Hz

Dimensions

20" x 9" x 10" (50.8 cm x 25.4 cm x 27.9 cm) without nozzles.

Weight

18.5 lbs. (8.4 kg.).

Standard Accessories for the EL404

Description	Bio-Tek Part Number
Drip Pan	4032038
Mirror	4032029
Operator's manual	4101000
Power Cord set	75011
Allen wrench for removal of tube manifold	48118
Stylus for cleaning dispense and aspiration tubes	4030555
Dust Cover	4032053
Warranty card	—
Certificate of Compliance and Calibration	98022
Shipping Document Kit	94075
Spare fuse, North American (Qty 1) 2.0A Slow Blo 1.25 x .25"	46078
Spare fuse, European (Qty 2) 2.0A Slow Blo 5 x 20 mm	46079

Optional Accessories for EL404 Washers

Description	Bio-Tek Part Number
Vinyl plate adapter for stabilizing PVC plates	3070086
Aerosol cover	4030506
Complete pressure delivery kit	4030517
Fluid reservoir bottles/tubing	4030523
• Fluid reservoir bottle (wash)	4030521
• Fluid reservoir bottle (rinse)	4030536
• Tubing	4030514
• 110V pressure-delivery pump	4030510
• Power cord	75011
• Bottle stand for 2 bottles	4032066
Complete gravity feed delivery kit (not including vacuum system)	4030516
Fluid reservoir bottle (2 supplied)	48106
Dispense set	4020070
Complete vacuum system kit	4030518
Vacuum bottles and tubing	4030524

• Vacuum tubing set	4030515
• 4-liter vacuum bottles and caps (2 supplied)	4030522
• Bottle stand for 2 bottles	4032066
• 110V vacuum pump assembly	4030512
Additional *U*-bottom microplate adapter	4032054 and 4032037

Combined pressure/vacuum kit	4030550
Pressure/vacuum module	4030542
Fluid reservoir bottles and tubing	4030523
• Fluid reservoir bottle (wash)	4030521
• Fluid reservoir bottle (Dispense)	4030536
• Tubing	4030514
• Bottle stand for 2 bottles	4032066
Power cord	75011
Vacuum bottles and tubing	4030524
• Vacuum tubing set	4030515
• 4-liter vacuum bottle & cap (2)	4030522
• Bottle stand for 2 bottles	4032066
Connector	49418
Optional Vacuum gauge	4030551

Replacement Parts

Male Bottle Top Connectors	49314
Female Bottle Top Connectors	49313

International Optional Accessories

The international optional accessories are the same as the domestic optional accessories except as noted in this section.

Description		Part Number
Complete pressure-delivery kit		4030528
• Fluid reservoir bottles and tubing		4030523
• 240 V pressure-delivery module		4030519
Complete vacuum-system kit		4030529
• Fluid reservoir bottles and tubing		4030524
• 220 to 240 V vacuum pump assembly		4030520
Combined Pressure/Vacuum Kit		4030553
• Pressure Vacuum Module		4030543
• Fluid Reservoir Bottles and Tubing		4030523
• Vacuum Bottles and Tubing		4030524
• Power Cord set:	(UK)	75012
	(German)	75010
	(Aust.)	75013

Setup Recommendations: User-Supplied Accessories

The EL404 has been constructed to work with a wide variety of accessories so that existing equipment may be used. However, when the EL404 is used with accessories other than those supplied by Bio-Tek, the intended performance of the washer may be altered.

The following sections discuss in detail what the EL404 requires and what is possible with different equipment configurations.

Pressure-Delivery Systems Requirements

Pressure Range/Gravity Feed Height:

- Any pressurized air source should work if the pressure is stable between **0.86 and 6.0 psi**. Above 6.0 psi, some splashing out of the microwells may occur.

- The EL404 is **calibrated at 1.5 psi** with 3/8" I.D. tubing, and accurate volumes are maintained at a pressure range of approximately 1.0 to 2.0 psi. Higher pressures result in slightly greater dispense volumes and lower pressures result in smaller dispense volumes than the volumes shown on the washer's display.

  These resulting volumes at other pressures are very repeatable, though they may differ slightly from what is displayed. As an example, if 250 microliters is displayed and 3.0 psi is the pressure, around 275 to 290 microliters will be dispensed. Whatever is dispensed in this range is, however, very repeatable.

  Run the washer **at a pressure appropriate to the assay.**

Pressure Range	Height
Stopcocks, valves and fittings have a minimum 1/4" (6.4 mm) inside diameter. 3/8" (9.5 mm) ID tubing; 1/8" (3.2 mm) wall thickness.	
0.86 to 6.0 psi	26 inches to 170 inches above working surface
Calibration Setting	
1.5 psi	47 inches above working surface
Recommended Pressure / Height for Most Assays	
1.5 psi to 3.0 psi	44 inches to 88 inches above working surface
Lightly Bound Reagents (some cellular assays, etc.)	
1.0 to 1.5 psi	30 inches to 44 inches above working surface
Some HIV, Hepatitis, Sticky Assays, Other Cellular Assays	
3.0 to 6.0 psi	

- Systems with bottles not placed at the same level as the washer require adjusting the pressure to compensate. If the bottles are lower than the washer, the pressure needs to be increased 0.4 psi for every 12" lower; if the bottles are higher than the washer, the pressure needs to be decreased 0.4 psi for every 12" higher.

  Example: The bottles are placed on the floor, 3 feet below the washer and 1.5 psi is the desired pressure at the inlet port of the washer. The pump pressure should be 1.5 psi + 1.2 psi = 2.7 psi.

Fluid Reservoirs

- The bottles need to hold enough fluid to prime the system. Wash a few microplates, and perform Overnight Maintenance. Two liters would be the absolute minimum if the operator is clever about minimizing fluid use. Four liters is optimum and that is what is supplied by Bio-Tek.

- The bottles must withstand the specified pressure requirements.

- Larger bottles also can be used for dispensing reagents only. The larger *Nalgene* bottles are recommended because they can withstand pressure. Bio-Tek's bottle caps with the quick disconnects can also be used with Nalgene bottles. We advise the following:

    - Ensure that the cap fits tightly on the bottle.

    - Undo the clamp on the underside of the cap and replace the shorter tube for the 4-liter bottle with a longer tube that descends to the bottom of the new bottle, is slit at the end, has 3/8" (9-10 mm) ID, and 5/8" (16 mm) OD.

    - Fill the bottle, screw on the cap, and try using it.

Tubing

- The tubing should have a minimum inside diameter of 3/8" (9 — 10 mm), a minimum outside diameter of 5/8" (16 mm) or 1/4" (6.4 mm) and a minimum outside diameter of 1/2" (12.7 mm).

- Tubing with narrower inside diameters, such as 1/4", require higher pressure settings to obtain the proper flow. Tubing with thinner walls may not be able to withstand the pressure.

- Valves or fittings should have a minimum 1/4" (6.4 mm) inside diameter.

- The tubing should be transparent to facilitate priming and to observe if the lines are full.

Connections:

- All connections should be clamped to prevent leaks.

Gravity-Feed System Requirements

Fluid Reservoirs

- The bottles need to hold enough fluid to prime the system. Wash some microplates, and perform OVERNIGHT MAINTENANCE. This fluid level should be measured from above the exit port of the washer.

- The bottles should not be collapsible. As the bottles collapse, they supply negative pressure.

- Exit ports should have a minimum inside diameter of 1/4" (6 mm) and should be pointed down to prevent kinking in the tubing.

Bottle Height Requirements

- The bottles should be raised to a height between 26 and 167 inches (66 to 424 cm) above the working surface of the washer. This corresponds to between 24 and 165 inches above the fluid inlet.

  To ensure adequate fluid level height, this distance should be measured between the working surface of the washer and the top of the shelf above the washer upon which the bottles are resting.

- The recommended fluid level is 44 inches (111 cm) above the working surface of the washer. This corresponds to 42 inches above the fluid inlet. This is the fluid level at which the EL404 is calibrated.

- Fluid levels less than 44 inches above the working surface will cause slightly less volume to be delivered and levels much greater than 44 inches above the working surface will cause slightly greater volumes to be delivered than the values displayed on the washer. As an example, if 250 microliters is displayed, a fluid level of 26 inches will dispense about 230 microliters.

- The shelf upon which the reservoirs are placed should be able to support at least 100 lbs. (45.5 kg.).

Tubing:

- The tubing should have a minimum inside diameter of 3/8" (9 — 10 mm) and a minimum outside diameter of 1/2" (12.5 — 13 mm). The wall thickness therefore has a minimum of 1/16" (1.5 — 2 mm).

  Thinner tubing is **not** recommended because the bottles must be raised to compensate. Raising the bottles to the minimum height and using thinner tubing than recommended will not provide enough flow for the system to operate. Wider tubing will increase the flow; usually this is beneficial.

- The tubing should be transparent to facilitate priming and to observe if the lines are full.

- Valves or fittings should have a minimum 1/4" (6 mm) ID.

Connecting to EL402 Gravity-Feed Accessories:

- The EL404 can be connected to the dispense set and bottles supplied with Bio-Tek's EL402 or EL403 washer.

Vacuum Systems Requirements

Vacuum Force

- The displacement ranges from 10 inches of Hg at 1.0 cubic feet/ minute to 20 inches of Hg and higher at 0.3 cubic feet/minute depending on the type of vacuum bottles used.

- The higher the force, the stronger the collection bottles should be. Some plastic bottles may collapse if the vacuum force is too high.

- Some assays require lower vacuum force. Cellular assays may require very low forces which are obtainable with the proper vacuum gauge or relief valve attached to the vacuum pump.

- Some ELISA assays have weakly bound reagents; these may also require low vacuum forces.

- The vacuum pumps supplied by Bio-Tek are equipped with relief valves which reduce the vacuum to about 18 inches of Hg. Vacuum gauge / regulator assemblies are available for external vacuum regulation.

Pumps

- Any pump or vacuum source such as house vacuum can be used as long as the vacuum can supply a stable flow between the stated limits. More powerful vacuum pumps can be used as long as the waste containers can withstand the force.

- Some collapsible bottles may require anti-collapse rings and a relief valve on the pump.

Collection Bottles

- Any set of collection bottles can be used if the customer supplies the tubing. The bottles must be strong enough for the vacuum force.

- Bio-Tek's plastic heavy-duty polypropylene bottles (labeled as such), can withstand vacuum force of up to 28 inches of Hg.

- Bio-Tek's thick-walled 4-liter glass flasks can withstand a vacuum force of up to 26 inches of Hg.

- Some larger plastic bottles, such as those supplied by *Nalgene*, are not recommended because they collapse under vacuum.

Vacuum Tubing

- The tubing must have a minimum inside diameter of 3/8" (9.5 mm) and a minimum wall thickness of 1/8" (3.2 mm). Thinner walled tubing will collapse under vacuum force.

- A second piece of tubing from the vacuum source (1/4" (6.5 mm) ID and 1/8" (3.2 mm) or greater wall thickness) must be connected to the vacuum sensing switch located in the lower right corner of the EL404 rear panel.

  Bio-Tek's tubing has a Y-connector to connect both the vacuum sensing tube and the main vacuum line to the pump.

Connecting to EL402 Vacuum Accessories

- The EL404 can be connected to the Bio-Tek EL402 glass flasks and tubing provided that: a Y-connector is installed in the system before the pump, and that a second tube connects the pump with the vacuum switch on the EL404 via this Y-connector.

Preventing Pump Damage

- The primary collection bottle should be emptied before the waste gets to the top of the bottle so that fluid is not aspirated into the second bottle.

- Often, an in-line filter such as a *Gelman* filter is installed between the last bottle and the pump.

- Do not allow fluid to be aspirated into the vacuum pump. Prevent this by installing an in-line filter larger than 2 microns in the vacuum line. If fluid is brought into the vacuum pump, call Bio-Tek's Technical Assistance Center for instructions.

Optimizing the System

- Once the system is working satisfactorily, you can shorten or lengthen the tubing depending on your space requirements. The vacuum system can, of course, be placed on the floor.

APPENDIX A

Computer Control

This appendix provides basic information on using the EL404's computer control features. If you are developing custom software for use with the EL404, you may require additional technical information and training. Contact Bio-Tek for additional support information at 1-800-451-5172. Outside the U.S., please call 802-655-4740.

RS232 Control of the EL404

The EL404's RS232 bi-directional interface allows a host computer to control the operation of the EL404 washer.

Section 1: Requirements:

To initiate computer control of the EL404, the following requirements must be met:

- The host computer must have an available serial (RS232) port (COM 1-4).

- A serial (RS232) cable must connect the host computer and the EL404. The EL404's serial port is configured as equipment data communications, and has a 25-pin female connector. A cable is available from Bio-Tek (P/N 75031) to allow connection to the host computer's 9-pin serial port. This cable provides additional connections that are not required by the EL404. *Figures A-1* and *A-2* illustrate connections between the computer and washer.

Earlier models of the EL404 used a 25-pin male connector for data communications. To upgrade an earlier model, contact Bio-Tek Service for a conversion kit.

- Communications software capable of sending and receiving binary data via an RS232C port must be loaded on the host computer in order to interface with the EL404.

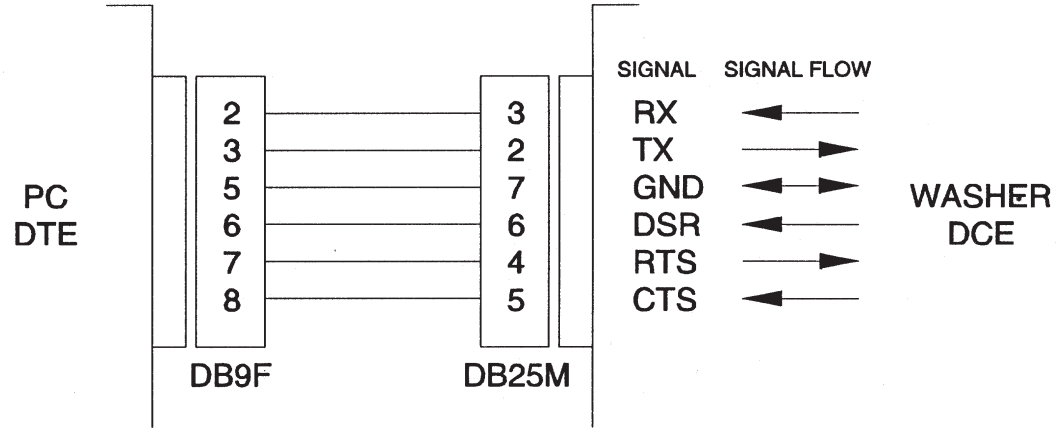


Figure A-1: Required Connections, 9-pin PC port

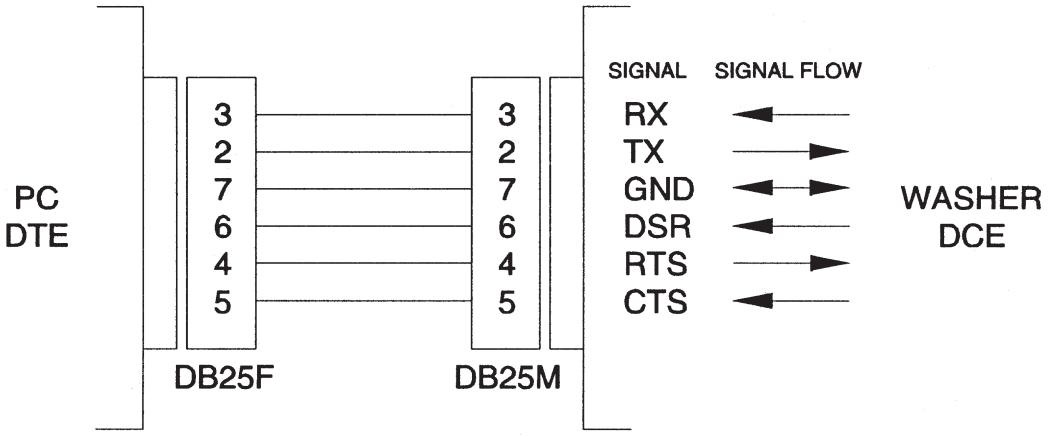


Figure A-2: Required Connections, 25-pin PC port

Section 2: RS232 Communications Parameters

The host computer and the EL404 must be set to the same communications parameters (Baud Rate, Parity, Data and Stop Bits).

On the EL404, Parity, Data and Stop bits are fixed at No Parity, 1 Stop bit, and 8 Data bits, and are not user configurable. However, the user may change the Baud rate on the washer. Choices are 1200, 2400 and 9600.

The commuications software used on the host computer must be set up with the same communications parameters as the EL404.

The DOS **Mode** command can be used to set the host computer's COM port parameters. For example, to provide compatibility between the computer's COM1 port and the EL404 at a 9600 baud rate, enter:

mode com1:9600,n,8,1

Refer to the DOS manual for information on the Mode command.

Setting the EL404's Baud Rate

1. **Turn on the main power switch, or move to the READY display by repeatedly pressing the washer's STOP/ESC key.**

   READY will appear on the EL404 display.

2. **Press the MAINT/HEIGHT key.**

   DAY MAINTENANCE appears on the display.

3. **Press the UP option key until the display shows RS232 PARAMETERS.**

4. **Press the LEFT CURSOR key until the desired baud rate is displayed.**

5. **Press the STOP/ESC key to return to the READY screen.**

Section 3: Initiating Computer Control

1. **Connect the computer and the EL404, using the appropriate cable.**

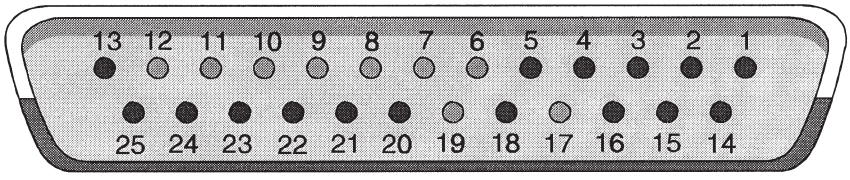
2. **Connect one end of the cable to the computer's serial port, and the other end to the EL404's 25-pin RS232 port.**

3. **Power up the computer and the EL404. The EL404 will display the following power up message:**

READY
Select a Function

4. **Start the communications program on the host computer, and initiate computer control of the washer by sending a command string.**

   A good choice is the Day Maintenance command, which will cause the washer carrier to move if successfully executed. Commands are discussed in Section 4.



EL404 SERIAL PORT PIN-OUT

2 - TX **(input)**
3 - RX **(output)**
4 - RTS **(input)**
5 - CTS **(output)**
6 - DSR **(output)**
7 - GND **(common)**

Section 4: Commands

The table that follows lists the most commonly used commands and their associated command strings for transmission to the EL404. The strings are shown in hexidecimal notation in the sequence that they are to be transmitted, in two-byte serial words. The strings in the tables that follow contain blank spaces for readability. However, **no spaces** are allowed in strings entered for transmission.

Command Name	Command Description	Command String, binary values in hexidecimal notation
Day maintenance, deep. Aspirate at 115.	Executes the day maintenance cycle using the highest aspiration level.	00 00 EE 03 02 00 73 00 73 00
Day maintenance, nominal. Aspirate at 100.	Executes the day maintenance cycle using a mid aspirate level.	00 00 EE 03 02 00 64 00 64 00
Day maintenance, shallow. Aspirate at 85.	Executes the day maintenance cycle using a shallow aspirate level.	00 00 EE 03 02 00 55 00 55 00
Overnight maintenance, deep. Aspirate at 115.	Executes the overnight maintenance cycle using a deep aspiration level.	00 00 EF 03 02 00 73 00 73 00
Overnight maintenance, nominal. Aspirate at 100.	Executes the overnight maintenance cycle using a mid aspirate level.	00 00 EF 03 02 00 64 00 64 00
Overnight maintenance, shallow. Aspirate at 85	Executes the overnight maintenance cycle using a shallow aspirate level	00 00 EF 03 02 00 55 00 55 00
Run wash 1	Execute the wash program stored as number 1 in EL404 memory	00 00 EA 03 02 00 00 00 00 00
Run wash 2	Execute the wash program stored as number 2 in EL404 memory	00 00 EA 03 02 00 01 00 01 00
Run wash 3	Execute the wash program stored as number 3 in EL404 memory	00 00 EA 03 02 00 02 00 02 00
Run wash 4	Execute the wash program stored as number 4 in EL404 memory	00 00 EA 03 02 00 03 00 03 00
Run wash 5	Execute the wash program stored as number 5 in EL404 memory	00 00 EA 03 02 00 04 00 04 00

Command Name	Command Description	Command String, binary values in hexidecimal notation
Run wash 6	Execute the wash program stored as number 6 in EL404 memory.	00 00 EA 03 02 00 05 00 05 00
Run wash 7	Execute the wash program stored as number 7 in EL404 memory.	00 00 EA 03 02 00 06 00 06 00
Run wash 8	Execute the wash program stored as number 8 in EL404 memory.	00 00 EA 03 02 00 07 00 07 00
Run wash 9	Execute the wash program stored as number 9 in EL404 memory.	00 00 EA 03 02 00 08 00 08 00
Run wash 10	Execute the wash program stored as number 10 in EL404 memory.	00 00 EA 03 02 00 09 00 09 00
Set dispense height, deep. Height is 90.	Set the dispense height at a deep level.	00 00 EB 03 02 00 5A 00 5A 00
Set dispense height, nominal. Height is 60.	Set the dispense height at a mid position.	00 00 EB 03 02 00 3C 00 3C 00
Set Dispense height, shallow. Height is 30.	Set the dispense height at a shallow position.	00 00 EB 03 02 00 1E 00 1E 00
Prime deep. Aspirate at 115.	Execute a prime cycle using a deep aspiration level.	00 00 EC 03 02 00 73 00 73 00
Prime, nominal. Aspirate at 100.	Execute a prime cycle using a mid aspiration level.	00 00 EC 03 02 00 64 00 64 00
Prime, shallow. Aspirate at 85.	Execute a prime cycle using a shallow aspiration level.	00 00 EC 03 02 00 55 00 55 00
Empty and prime, deep. Aspirate at 115.	Execute an empty & prime cycle using a deep aspiration level.	00 00 ED 03 02 00 73 00 73 00
Empty and prime, nominal. Aspirate at 100.	Execute an empty and prime cycle using a mid aspiration level.	00 00 ED 03 02 00 64 00 64 00
Empty and prime, shallow. Aspirate at 85.	Execute an empty and prime cycle using a shallow aspiration level.	00 00 ED 03 02 00 55 00 55 00

Command Name	Command Description	Command String, binary values in hexidecimal notation
Rinse, deep. Aspirate at 115.	Execute a rinse cycle using the highest aspiration level.	00 00 F0 03 02 00 73 00 73 00
Rinse, nominal. Aspirate at 100.	Execute a rinse cycle using a mid aspiration level.	00 00 F0 03 02 00 64 00 64 00
Rinse, shallow. Aspirate at 85.	Execute a rinse cycle using the lowest aspiration level.	00 00 F0 03 02 00 55 00 55 00
Aspirate, deep. Aspirate at 115.	Execute the aspirate cycle, using a deep aspirate level.	00 00 F1 03 02 00 73 00 73 00
Aspirate, nominal. Aspirate at 100.	Execute the aspirate cycle, using a mid aspirate level.	00 00 F1 03 02 00 64 00 64 00
Aspirate, shallow. Aspirate at 85.	Execute the aspirate cycle, using a shallow aspirate level.	00 00 F1 03 02 00 55 00 55 00
Dispense, 200. Aspirate at 100.	Execute a dispense cycle delivering 200 ul of wash fluid at a shallow dispense height.	00 00 F2 03 04 00 2C 01 C8 00 64 00
Dispense, 250. Aspirate at 100.	Execute a dispense cycle delivering 250 ul of wash fluid at a shallow dispense height.	00 00 F2 03 04 00 2C 01 C8 00 64 00
Dispense, 300. Aspirate at 100.	Execute a dispense cycle delivering 300 ul of wash fluid at a shallow dispense height.	00 00 F2 03 04 00 91 01 2C 01 64 00
Dispense, 350. Aspirate at 100.	Execute a dispense cycle delivering 350 ul of wash fluid at a shallow dispense height.	00 00 F2 03 04 00 C3 01 5E 01 64 00
Dispense, 400. Aspirate at 100.	Execute a dispense cycle delivering 400 ul of wash fluid at a shallow dispense height.	00 00 F2 03 04 00 F5 01 90 01 64 00
Set mode, demo	Place the washer in demonstration mode.	00 00 F4 03 01 00 59 00 59
Set mode, normal	Place the washer in normal washer mode.	00 00 F4 03 01 00 4E 00 4E

Section 5: Status and other return characters

When a command string has successfully executed a function on the washer, the microplate carrier returns to the home position, and a status message is sent from the washer to the host computer.

The status message either indicates that the wash operation was successful, or not.

If no error occurred, a status code of 0006 will be returned. If an error state was encountered, the EL404 will return a status code depending on the error. The most common error codes are detailed in the table that follows.

Error codes are listed as decimal values but are transmitted as four-character hexidecimal values, two characters per serial word.

Message Transmission Rate

The host computer's software is required to process the return status from each command sent prior to issuing another command.

Returning the Washer to Manual Mode

When a command string has successfully executed a function on the washer, the EL404 remains in computer control mode.

1. **Press the STOP/ESC key on the EL404 to return the washer to manual mode and discontinue computer control.**

Error Codes

Status Decimal	Status HEX coded binary	Name	Description
0006	0600	ACK	No error. Returned for every successful command
1000	E803	Unknown error	Error in the format of the command message or serial communications error
1001	E903	Inadequate vacuum	
1002	E903	Inadequate flow	
1003	EB03	No plate sensed	
1004	EC03	Plate crashing into tubes	Positional error
1005	ED03	Home switch malfunction	Positional error
1006	EE03	Dispense/overflow height greater than aspiration depth	
1007	EF03	Internal Status	
1008	F003	Internal Status	
1009	F103	Valve Leak Error	Not used
1010	F203	User Intervention	The user pressed STOP/ESC in the middle of a command.

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