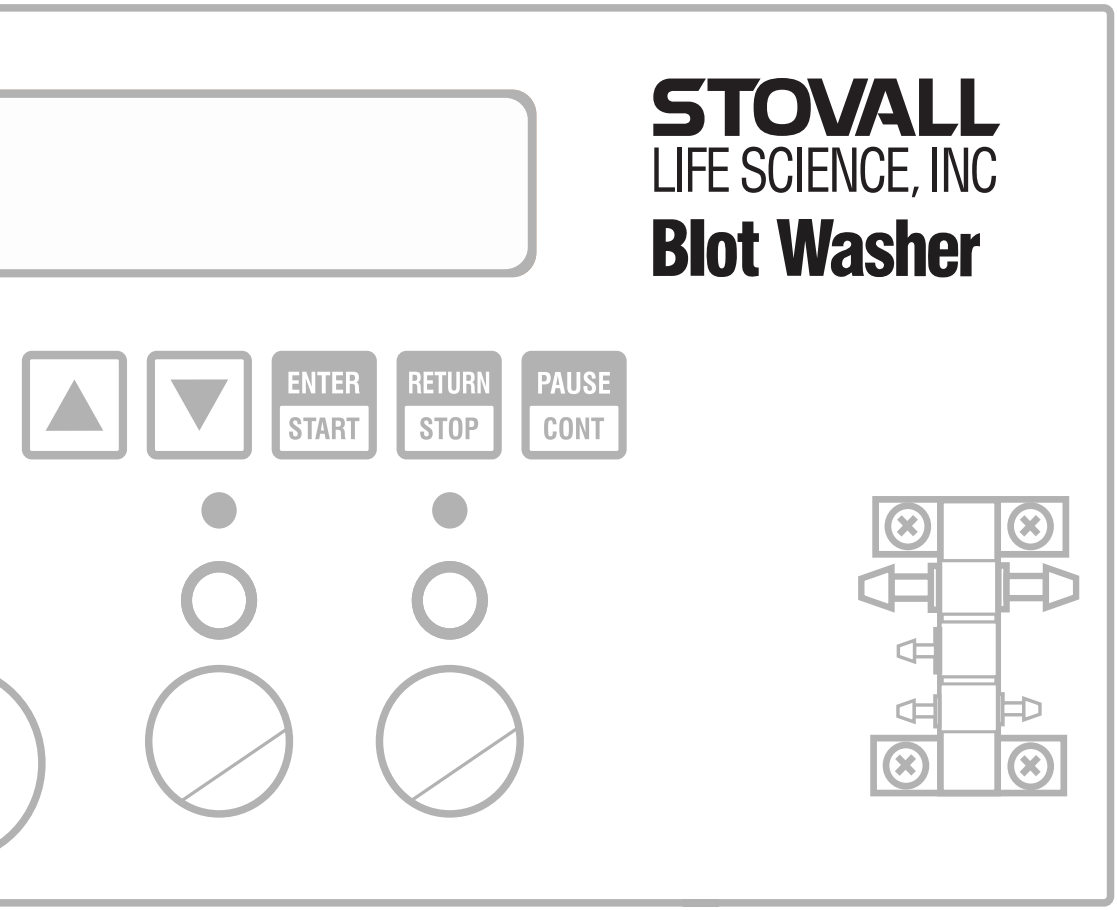

STOVALL
LIFE SCIENCE, INC

Blot Washer

Operator's Manual

For Automated Western Blot Processing

Notice

The information contained in this document is subject to change without notice.

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Chapter 1: System Familiarization and Assembly

Placement in the Laboratory

Place the instrument away from sinks or other sources of water that pose a shock hazard. Do not locate the Blot Washer where the power cord will be walked on or exposed to water or chemical spills.

Warning: Do not operate around flammable liquids or gases.

Electrical Considerations

For personal safety the Blot Washer must be properly grounded. The power cords have a three-prong, grounded plug that mates with a standard three-prong, grounded wall receptacle to minimize the possibility of electric shock. The user should have the wall receptacle and circuit checked by a qualified electrician to be assured that the receptacle is properly grounded. Where a two-prong receptacle is encountered, it is the responsibility of the user to replace it with a properly grounded three-prong wall receptacle.

Warning: Do not under any circumstances, cut or remove the third ground prong from the power cord. Do not use a two-prong adapter plug.

Current Requirements

Current demand for the Blot Washer is modest, but it should be added to other current demand on the circuit to make sure total current demand does not exceed the rating of the fuse or circuit breaker in use. Current demand for the various Blot Washer models are listed below.

Blot Washer Model	Current Demand
115V	< 0.5 Amps
230V	< 0.25 Amps

Caution: Be sure the line voltage is of the same value specified on the nameplate located on the back panel.

Fuse Replacement

Warning: Before replacing a blown fuse, turn all switches to the OFF position and unplug the instrument.

Figure 1 shows the location of the fuse in a small fuse drawer on the back panel of the Blot Washer. A spare fuse is also located in this drawer. Should the fuse blow, replace with a new fuse of the same value. To remove a fuse, place the tip of a slot blade screwdriver into the top of the fuse drawer and pry open.

Blot Washer Model	Fuse Description
115V	5 mm x 20 mm, 500 mAmp
230V	5 mm x 20 mm, 250 mAmp

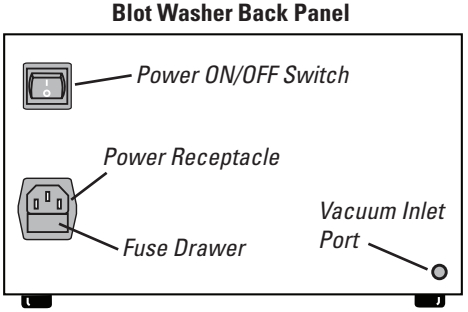


Figure 1. Blot washer back panel showing location of the ON/OFF power switch, power receptacle, fuse drawer, and vacuum port.

Connecting Power

Connect the power cord to the power receptacle on the Blot Washer back panel and connect the power cord plug to a wall or bench outlet.

System Overview

Hardware Familiarization

The Stovall Blot Washer is a straightforward liquid delivery and aspiration instrument. An internal pump supplies positive pressure for liquid delivery and house vacuum aspirates waste liquids from blot containers. Air pressure from the internal pump is directed through the **blue pressure port** located on the left side of the Blot Washer (Figure 2). The vacuum inlet is located on the back panel of the instrument (Figure 1) and the **black vacuum outlet port** is beneath the blue air pressure port on the left side of the instrument front panel.

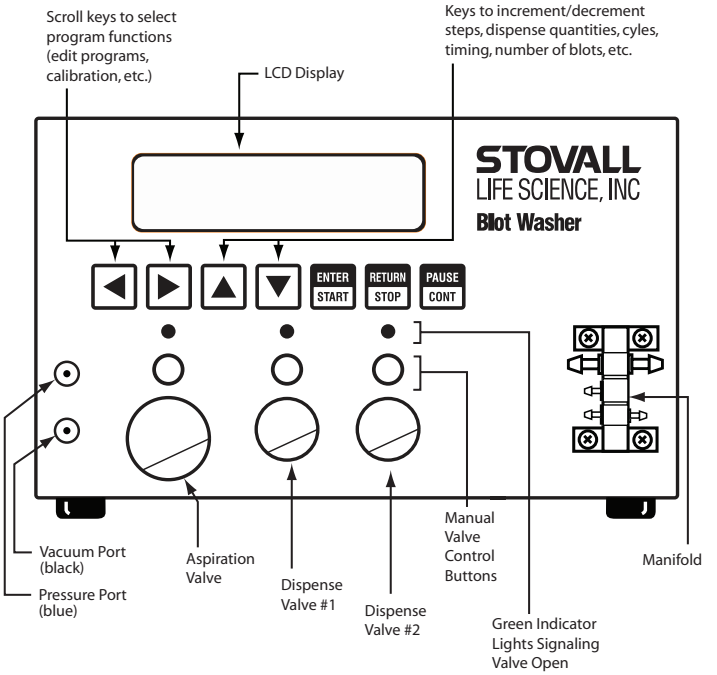


Figure 2. Major components on the Blot Washer front panel.

Software wash programs operate three solenoid valves to control the flow of liquids. The normal condition of all valves is closed, where by the flow of liquids is impeded by pinching the silicone tubing closed. From left to right on the front panel (Figure 2) the valves are:

- **Aspiration Valve** (larger) controls the aspiration of liquids to the waste bottle.
- **Dispense Valve #1** controls the delivery of wash buffer from the large wash bottle.
- **Dispense Valve #2** controls reagent delivery from the 140 ml syringe or a second delivery reservoir.

During operation, the internal pump pressurizes the Wash Bottle (Figure 3) through an air pressure line. When a new wash cycle starts, Dispense Solenoid Valve #1 opens and the pressure in the Wash Bottle dispenses wash buffer from the Wash Bottle to the blot container.

The vacuum source is used to lower the air pressure in the Waste Bottle. During a waste removal, the Aspiration Solenoid Valve (Figure 2) opens and waste liquid is aspirated from the blot container, and flows into the Waste Bottle.

When a wash program is configured, the user enters how many of these dispense/aspiration cycles there are in a given wash step, how much wash buffer is dispensed, and how long the liquid remains in the blot container before aspiration.

Dispense Solenoid Valve #2 adds versatility by allowing a second delivery container to be connected (Figure 5). Similar to the Wash bottle, this second container is pressurized by the pump and liquid is dispensed to the blot container when Dispense Solenoid Valve #2 is opened under program control. This second delivery container can be used to dispense a second wash buffer or secondary antibodies.

Each of the solenoid valves has a circular button above it. These buttons manually open the solenoid valves and operate the valves independently of programmed sequences. Manual control is most commonly used when tubes are flushed during cleaning or during calibration. When a solenoid valve is open, under program or manual control, the indicator light above the manual control button is illuminated.

Software Familiarization

Creating programs for a variety of wash sequences is described in Chapter 2 and additional software functions for calibration are discussed in Chapter 3. Before connecting tubing and filling

reservoir bottles, remove all port plugs, turn on the power switch, and use the instructions in Chapters 2 and 3 to familiarize yourself with the keypad and general software operation.

The most important keys to learn are the ◄ and ► keys for navigation through menus and programs, and the ▲ or ▼ keys for choosing options or changing numbers. The ► key is especially important for navigation when editing programs. The other three keys on the keypad are used to start, stop and pause wash programs.

Tube Routing

The Blot Washer, reservoir bottles, and blot containers can be placed in many different configurations depending on available lab space. Lengths of supplied tubing are initially generous enough to accommodate different configurations. Once an optimal configuration has been determined for your lab, you may want to shorten excessively long tubes for a more organized, orderly appearance.

Figure 3 shows tubing connections for a standard configuration that can be used to wash blots in one blot container. Tube routing is shown for the wash bottle, waste bottle and vacuum source, Blot Washer, and a single blot container connected to the Blot Washer using the single dispensing harness.

Color Coding

Connectors on the wash bottle, waste bottle, and Blot Washer are all color coded to facilitate making the correct connections. Connecting each end of a tube to the same color connector will lead to correct connections in most cases. The color codes have the following meanings:

Black: vacuum source
Blue: air pressure source
Orange: liquid waste
Green: liquid dispensing

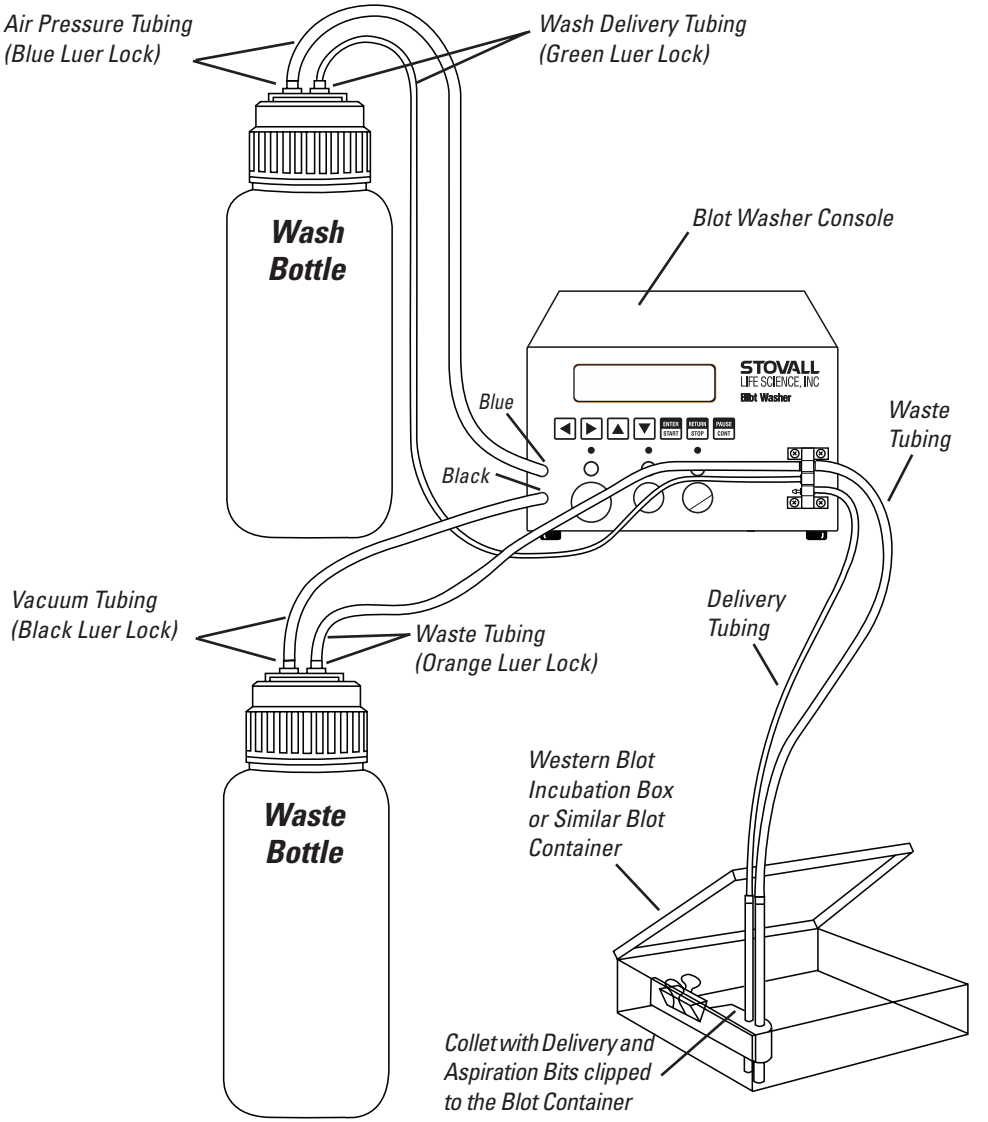


Figure 3. Standard tubing configuration for one delivery (wash) bottle and one blot container. Connector color codes are shown.

Connecting the Waste Removal System

Thick-walled, 1/4" i.d. tubing has been supplied for connecting a house vacuum source to the black vacuum port (Figure 1) on the back panel of the Blot Washer. Alternatively, this tubing can be used to connect the Accessory Vacuum Pump.

The vacuum passes though the console and is available at the black connector on the lower left side of the Blot Washer front panel (Figure 2). Connect the black front panel vacuum connector to the matching black connector on the Waste Bottle. Use the orange connector to connect waste tubing from the Waste Bottle to the top connector on the left side of the manifold (Figure 3). The waste tubing should also be passed through the large Aspiration Solenoid Valve. Insertion of tubing in solenoid valves is described later in this chapter. Finally, the waste line is completed by connecting one end of the single dispensing harness waste tube to the right side connector on the manifold, and the other end to the aspiration bit attached to the blot container. Make sure the bottom of the aspiration bit touches the bottom of the blot container and the beveled side of the aspiration bit faces the wall of the container. (The aspiration bit suctions up the blot itself if the beveled side points to the middle of the blot container.)

Connecting the Wash System

For best performance, place the Wash Bottle above the blot container. Begin by connecting air delivery tubing from the blue luer lock on the left side of the Blot Washer front panel (Figure 2) to the blue luer lock connector on top of the Wash Bottle. Next connect a wash delivery tube (smaller than the air delivery tubing) from the green luer lock on top of the Wash Bottle to the middle connector on the left side of the manifold (Figure 2). The wash delivery tubing should pass through the Dispense Valve #1. Insertion of tubing in solenoid valves is described below. Finally, the wash

delivery system is completed by connecting tubing from the single dispensing harness to the right side connector on the manifold and to the delivery bit in the blot container. The bottom of the delivery bit should touch the bottom of the blot container.

Inserting Tubing Into the Solenoid Valves

The solenoid valves are closed until opened by manual or program command. When placing the silicone tubing into a valve, or extracting it from a valve, manually open the valve by pressing the circular button above the valve as shown in Figure 4. Stretch the tubing enough to slip it into the valve slot.

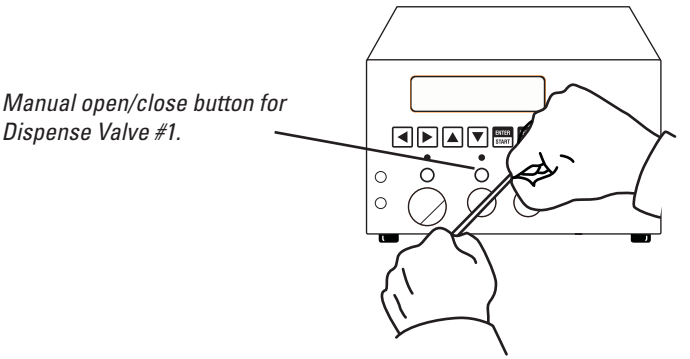


Figure 4. Tubing can be inserted in a solenoid valve by manually opening the valve, stretching and inserting the tubing, then closing the valve.

Position the tubing as needed by pulling it up or down, but only while the valve is open. The indicator light for the valve is turned on when the valve is open. When the tubing is positioned correctly, close the valve by pressing the manual open/close button again.

Checking for Leaks

After loading the containers with reagents in preparation for a run, make sure all container lids are tight and luer lock connections are secure. Chapter 3 describes a software function that indicates whether the system is properly pressurized. Low pressure (< 20 kPa) usually indicates a leak.

Using a Second Delivery Container

Figure 5 shows how to reconfigure the liquid delivery system for two delivery containers. In Figure 5, the second delivery container is the 140 ml syringe, but it could also be a second wash bottle for a configuration that delivers two different wash buffers. A typical application for a second container is to deliver secondary antibodies.

Notice that the air pressure provided by the Blot Washer's pump is divided with a "Y" connector in the tubing. The wash delivery tube from the Wash Bottle is routed through Dispense Valve #1, as before, but a second delivery tube from the syringe or second container is routed through Dispense Valve #2 and connects to the lower connector on the left side of the manifold. Connecting the second delivery tube to the lower connector has the important advantage of minimizing system contamination. When the wash delivery tube is connected to the upper connector of the liquid delivery part of the manifold, the inside of the manifold is cleaned throughout the program.

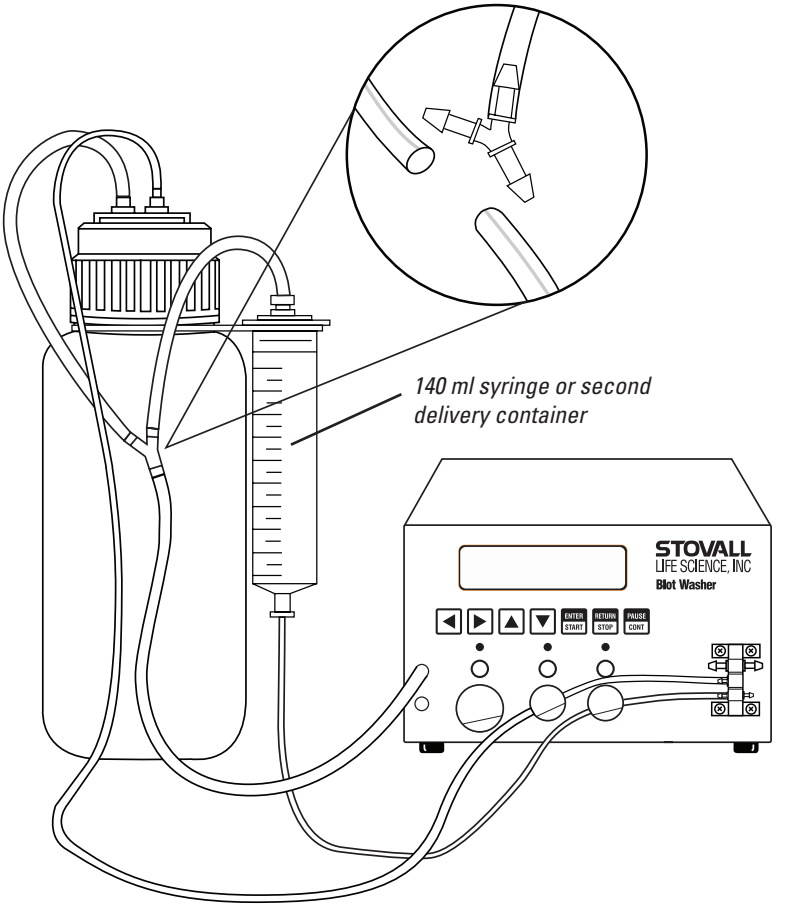


Figure 5. Tubing configuration for using the 140 ml syringe or other secondary delivery container (waste tubing not shown).

Cleaning the Blot Washer After Use

Cleaning Tubing and Containers

Cleaning of reagent containers and tubing is the primary maintenance requirement. To eliminate almost all potential maintenance problems, establish the habit of cleaning the Blot Washer immediately after use. Reagent residues are easy to clean up while they are fresh and still wet. There are two adverse consequences of leaving used reagents in the tubing for any length of time. The first is that common antibody dilution buffers (BSA) are excellent substrates for microbial growth. Second, over several days, microbial growth, dried protein, or buffer crystals can form in the tubing, making cleaning more difficult. The blot container should be clean for every experiment.

For best performance, clean the delivery and waste tubing with water immediately after each use. Disconnect the wash tubing from the green luer lock on top of the wash bottle. Manually open the dispense valve using the button above the valve and use a wash bottle to flow water through the wash tubing. Close the dispense valve when finished. The same procedure can be used to clean the waste tubing connected to the orange luer lock on the top of the waste bottle.

If you are running the same antibodies repeatedly, detergent cleaning is necessary only occasionally. To completely prevent cross contamination, residual protein/antibody in the lines can be cleared with standard laboratory detergent solutions (e.g. 0.5% Sparkleen, Nalgene L900 liquid cleaning solution) or with 0.5% sodium dodecyl sulphate (SDS), followed by water. Replace old or damaged tubing with new silicone tubing. If your programs contain only wash

steps (see Chapter 2) with buffers that do not contain protein, detergent cleaning is not necessary unless the Blot Washer is not going to be used for long periods of time (> 1 week).

Discarding Waste and Replacing Wash Buffer

Discarding Waste: Release the vacuum in the Waste Bottle by unscrewing the two luer locks that connect the tubing to the top of the bottle. Take the top off. Discard the waste. Replace the top and reconnect the tubing, observing the color coding — connect black to black and orange to orange.

Replacing Wash Buffer: Unscrew the two luer locks that connect the tubing to the top of the bottle. Take the top off. Replace the buffer. Occasionally, you should wash out the buffer bottle after many repeated uses, or if a different buffer is to be used. Replace the top and reconnect the tubing, observing the color coding — connect green to green and blue to blue.

Cleaning the Exterior Surface

Warning: Always unplug the power cord from the receptacle before cleaning the Blot Washer.

Warning: Do not immerse the Blot Washer in water or other liquid.

The exterior of the Blot Washer can be cleaned with a rag dampened with a solution of mild detergent. Rinse by wiping with a cloth dampened in water. Do not use scouring compounds, solvents such as acetone, benzene, carbon tetrachlorides, lacquer thinner, or alcohol to clean the exterior surfaces.

Chapter 2: Creating and Running Programs

Up to 20 programs, numbered 1 through 20, can be stored in the Blot Washer memory. With 1 to 4 individually configurable steps per program, Blot Washer has the flexibility to accomplish any typical blot washing task. Initially, each of the 20 programs are preprogrammed at the factory as described at the end of this section. As you create your own programs, you may want to develop a system to document each program for easy recall.

Keys You Should Be Familiar With

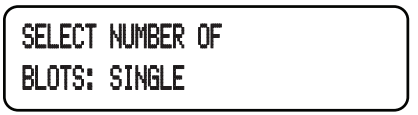
Most of the keystrokes in creating a program are used to toggle between available options for a program step, or to accept the current option and move to the next step.

▲ or ▼ – Toggles through available options for a step.

▶ – Accepts the current option and moves to the next step.

Powering On the Blot Washer

1) Turn on the power switch on the back of the Blot Washer control console.



The initial display is used to select the number of blots.

"Single" is always the correct choice unless the Quad Harness is being used. See *Using the Quad Harness* below to learn how to enter programs for washing 1 to 4 blots simultaneously.

Selecting the Program Number to Edit or Run

2) Press [▶] to accept "Single" for the number of blots.

```
SELECT PROGRAM
NUMBER: 1
```

Three actions are possible when this display is shown:

a) Press [▲] or [▼] to change the program number to any of the 20 programs.

b) Press [ENTER START] to run the specified program (see Starting a Program below).

c) Press [▶] to edit the specified program as described below.

Editing a Program

In this example, you'll make a few changes to program #1.

3) With program number 1 still displayed, press [▶] to edit the program.

```
EDIT PROGRAM 1
SELECT STEPS: 4
```

The [▲] and [▼] keys change the number of steps.

Each program can have up to four steps and each program step can be one of three types: DELAY, WASH, or INCUBATE.

Step Type	Description
DELAY	A time delay during which no actions are performed. A typical use is to add DELAY as the first step to allow for incubation of previously administered primary antibodies.
WASH	Performs a specified number of wash cycles. During each cycle, a specified volume of wash buffer is dispensed by opening a designated dispense valve and the blot is washed for a specified length of time before the wash buffer is aspirated.
INCUBATE	Aspirates tray contents, dispenses an antibody by opening a dispense valve, incubates for a specified interval, then aspirates at the end of the incubation time.

In addition to the actions in the description for each step type above, there are three additional actions that occur automatically and do not need to be programmed.

a) Unless Step #1 is a DELAY step, any liquid from the blot containers will be the aspirated at the start of Step #1. The amount of time for this initial aspiration is determined by the volume to be removed.

b) After the last cycle in a WASH step, a final dispense of the wash buffer occurs automatically so the blot will not dry out, even if left for hours.

c) Aspiration of liquids from the blot container(s) does not need to be programmed. Aspiration occurs automatically according to the amount of liquid(s) originally dispensed to the blot containers. Should the aspirating be too long or too short, calibration (Chapter 3) will correct the problem.

Any of the three types of steps can be assigned to a given step. Examples of typical programs are given below.

# Steps	Typical Steps
4	DELAY, WASH, INCUBATE, WASH
3	WASH, INCUBATE, WASH
2	WASH, WASH (two different wash buffers from different containers)
1	WASH

In our continuing example, program #1 will be edited to be a 4-step program.

4) Press [▶] with the number of steps set to "4".

```
EDIT P1  STEP 1
TYPE: DELAY
```

The top line of the display indicates this is step 1 of program number 1.

The [▲] and [▼] keys change the step type between DELAY, WASH, and INCUBATE.

5) Press [▶] to accept "DELAY" as the step type.

```
EDIT P1  STEP 1
DLY TIME: 01:00
```

The time format is HH:MM. Pressing [▲] increments by 15 minutes and [▼] decrements by 15 minutes. Pressing and holding [▲] or [▼] continuously increments or decrements the time while the key remains depressed.

6) Press [▶] to accept the current delay time and automatically advance to step 2.

```
EDIT P1  STEP 2
TYPE: WASH
```

WASH matches the step type needed for our program, but any of the step types can be slected.

7) Press [▶] to set the type to WASH and begin editing the step.

```
EDIT P1  STEP 2
WASH CYCLES: 5
```

Use the [▲] or [▼] keys change the number of cycles to match your protocol.

8) Press [▼] and then [▶] to set the number of wash cycles to 4.

```
EDIT P1  STEP 2
WASH VALVE: 1
```

Select WASH VALVE 1 OR 2 to dispense wash buffer from Dispense Valve #1 or Dispense Valve #2 on the Blot Washer front panel (see Figure 2, Chapter 1). Typically Dispense Valve #1 is used unless you want to dispense from a secondary delivery container.

9) Press [▶] to accept WASH VALVE #1.

```
EDIT P1  STEP 2
DISPENSE: 50 ML
```

The [▲] and [▼] keys increment the decrement and the DISPENSE Volume, respectively.

The DISPENSE volume (5 - 250 ml) is the volume of wash buffer that will be delivered during each wash cycle.

Important: The DISPENSE volume is the volume needed for one container, even if there are multiple containers. See *Using the Quad Harness* below.

10) Press [▶] to accept the current DISPENSE volume.

```
EDIT P1  STEP 2
WASH TIME: 01:00
```

Set WASH TIME to the time period (16 hour maximum) for one wash cycle. Total wash time will be WASH TIME multiplied by WASH CYCLES (step 7).

The WASH TIME format is HH:MM. Press ▲ or ▼ to increment or decrement by 1 minute. Press and hold ▲ or ▼ to continuously invrement or decrement at an accelerated rate.

11) Use the ▼ key to set the wash time to 5 minutes (00:05).

12) Press ▶ to finish the WASH step and advance to step 3.

The INCUBATE step type already matches the step type needed for this program example, so no changes are needed.

The third step will be used to dispense secondary antibodies to the blot container for incubation.

13) Press ▶ to accept the step type.

Typically, secondary antibodies for the incubation step are dispensed from the accessory syringe or a second reservoir bottle using Dispense Valve #2 on the Blot Washer front panel. This configuration will be assumed for this example program.

14) Press ▶ to accept Dispense Value #2.

The ▲ and ▼ keys increment and decrement the DISPENSE volume.

The DISPENSE volume (5 – 250 ml) is the volume of antibody solution that will be delivered to one container, even if the Quad Harness is being used to process multiple containers.

Note: The dispense volume entered should not exceed the volume of the 140 ml accessory syringe or other secondary delivery container.

15) Press [▶] to accept the DISPENSE volume.

```
EDIT P1  STEP 3
INCB TIME: 01:00
```

Set the incubation time (HH:MM) according to your protocol by pressing the [▲] and [▼] keys. Pressing and holding the arrow keys changes the incubation time at an accelerated rate. One hour is adequate for this example program.

16) Press [▶] to accept the INCUBATE step and advance to step 4.

```
EDIT P1  STEP 4
TYPE: WASH
```

This second and final wash step uses the same prompts for wash parameters (cycles, etc.) as the wash in program step two described earlier (7-10 above). Wash parameters would normally be entered to match your protocol, using the [▶] key used to advance through each of the parameters.

Important: After the last cycle in a wash step, a final dispense of the wash buffer occurs automatically so the blot will not dry out.

After entering the last parameter in the program (WASH TIME in this case), press [ENTER START] to return to the initial program selection display.

```
SELECT PROGRAM
NUMBER: 1
```

From this display, you can edit another program or run any of the 20 programs (see Starting a Program).

Starting a Program

1) Turn on the Blot Washer, if necessary.

```
SELECT NUMBER OF
BLOTS: SINGLE
```

2) Press ◄ to check the system pressure.

```
SYSTEM PRESSURE
23 KPA
```

This step is optional, but recommended, especially after changing tubing or filling liquid bottles.

20-23 KPA is typical. If system pressure is below 20 KPA, the system may need to be checked for leaks before proceeding (see Checking Pressure in Chapter 3).

3) Press ► to return to the initial display, which is used to select the number of blots.

```
SELECT NUMBER OF
BLOTS: SINGLE
```

SINGLE, 1 OF QUAD, 2 OF QUAD, 3 OF QUAD, or 4 OF QUAD can be chosen using the ▲ and ▼ keys.

4) Choose the number of blots and press ► to continue.

```
SELECT PROGRAM
NUMBER: 1
```

5) Select the program to run by pressing ▲ or ▼ until the desired program number is displayed.

6) Press ENTER START to run the specified program.

Pausing a Program

While a program is in operation it can be paused by pressing PAUSE CONT

The program number and current step are listed in the top line of the display. The second line indicates operation is paused.

Press PAUSE CONT to resume (continue) a paused program.

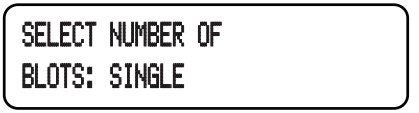
Stopping a Program

While a program is in operation it can be stopped by pressing RETURN STOP.

Quickly press RETURN STOP to again while the "Press Stop..." message is displayed. If you wait too long and the message changes, pressing RETURN STOP will just start the routine again.

Using the Quad Harness

If you process only one blot at a time, use the single dispensing harness to process one blot container. Set the number of blots on the initial display to "SINGLE" and then run the desired program to process the single container.



The Quad Dispensing Harness is used when you want a program to process 1 to 4 containers in the same way (Figure 7). For example, the following procedure processes four containers using program #2.

1) As described in Chapter 1, connect delivery/aspiration bits to all four containers and connect the Quad Harness to the right side of the manifold on the Blot Washer front panel.

2) Turn on the Blot Washer and wait until prompted for the number of blots.

```
SELECT NUMBER OF
BLOTS: SINGLE
```

3) Press [▲] four times until 4 OF QUAD is displayed, indicating that the same reagents will be dispensed to all four containers.

```
SELECT NUMBER OF
BLOTS: 4 OF QUAD
```

Note: When processing a single blot using the Quad Harness, always set the number of blots to 1 OF QUAD rather than SINGLE so that the delivery volumes are correct.

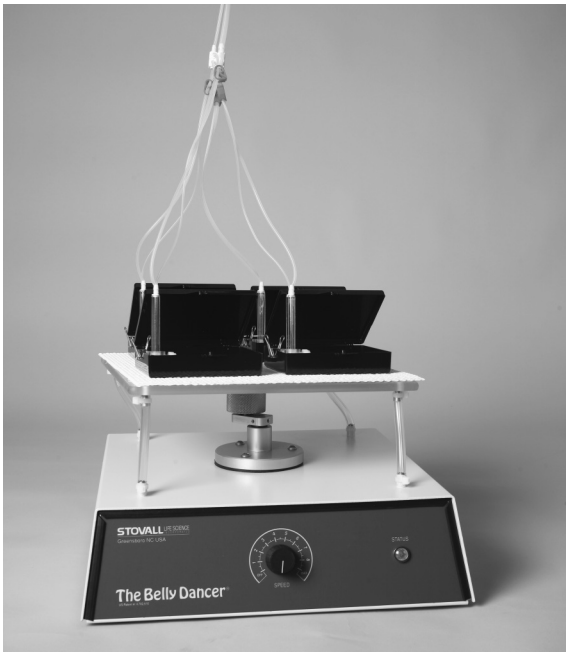


Figure 7. Quad harness connected to four Western Incubation Boxes on a shaker (The Belly Dancer, Stovall Life Science, Inc., Greensboro, NC).

4) Press [▶] to set the number of blots and continue.

```
SELECT PROGRAM
NUMBER: 1
```

5) Press [▲] once to increment the program number.

```
SELECT PROGRAM
NUMBER: 2
```

6) Press [ENTER START] to run program #2.

Scaling Up or Back

It is easy to change the number of containers processed. Only two modifications are needed to scale back the four-container procedure, described above, to process only two containers. First, clamp off the two unused delivery lines on the Quad Harness using the provided hose clamps. Second, run program #2 as described above, but choose 2 OF QUAD rather than 4 OF QUAD for NUMBER OF BLOTS.

Notice that no changes were needed to program #2 to compensate for volume of liquids dispensed. The dispense volume entered in a program step should always be that required for one container. For example, if the desired delivery for one container is 40 ml of wash buffer, the appropriate WASH step in the program should be set to dispense 40 ml. Blot Washer already knows that 40 ml should be delivered to each container if 2 OF QUAD, 3 OF QUAD, or 4 OF QUAD is chosen for NUMBER OF BLOTS.

Delivery rates for the Quad Harness are different from the single dispensing harness, but Blot Washer compensates for the difference when 1 OF QUAD, 2 OF QUAD, 3 OF QUAD, or 4 OF QUAD is chosen for NUMBER OF BLOTS. The duration that valves are open for delivery and aspiration is adjusted automatically when the stored program is started. These adjustments do not affect the wash or incubation duration. The wash or incubation time specified in the program starts only after liquid delivery is complete.

Default Program Descriptions

The 20 available programs are preprogrammed at the factory as described below. The editing procedure described earlier in this chapter can be used to modify these template programs to match your specific protocols.

Program #	Description
1-10	4-step DELAY, WASH, INCUBATE, and WASH programs.
11-13	3-step WASH, INCUBATE, and WASH programs.
14-16	2-step WASH, WASH programs.
17-19	1-step WASH programs.
20	A 4-step test program used during factory testing that can be modified in the same way as the other 19 programs.

Chapter 3: Calibration

The dispense rate and aspiration rate are factory calibrated. Volume of liquids delivered or aspirated is determined by the length of time the solenoid valves are open. For delivery, the pump maintains 23 KPA of pressure, which results in a dispense rate of approximately 2.5 ml/sec; aspiration of liquids by house vacuum is usually 5 ml/sec. Delivery rate varies when the liquid reservoirs are located above or below the blot containers; the aspiration rate can vary according to the strength of the house vacuum. (For best results, place the delivery container above the blot container(s).)

Calibration may be needed if the dispense volume is inaccurate or aspiration is incomplete or goes on too long. The simple calibration steps in this chapter adjust factory-set delivery and aspiration rates to appropriate values for your lab conditions.

Keys You Should Be Familiar With

The three calibration functions discussed in this chapter can be accessed by pressing ◀ (1x, 2x, or 3x) when SELECT NUMBER OF BLOTS is displayed initially. To return to the initial display, press ▶ (1x, 2x, or 3x) until SELECT NUMBER OF BLOTS is displayed again. You can also exit the calibration functions and start a program by pressing ENTER START.

Checking System Pressure

One factor that affects the dispense rate is the maintenance of air pressure in the liquid containers. System pressure should always be checked before proceeding to other calibration steps.

1) Press ◀ at the initial SELECT NUMBER OF BLOTS display to check the system pressure.

```
SYSTEM PRESSURE
23 KPA
```

20-23 KPA is typical. If system pressure is below 20 KPA, there is probably a leak: check the tightness of liquid container tops and tubing connections.

Calibrating Dispense Volume

If the system pressure is correct, as described above, but the volume dispensed is still inaccurate, the dispense volume needs to be calibrated. You will need a 100 ml graduated cylinder.

1) Fill the wash container at least half full of water or buffer.

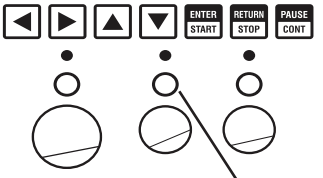
2) Connect the single dispensing harness, rather than the Quad Harness, if necessary.

3) Press ◀ once if system pressure is displayed.

```
TO CALIBRATE
DISPENSE 100 ML
```

4) Direct the delivery tube from Dispense Valve #1 to a 100 ml graduated cylinder.

5) Press the round button above Dispense Valve #1 to manually open the valve.



Manual open/close button for Dispense Valve #1.

6) Press the round button above Dispense Valve #1 to manually close the valve just as the 100 ml mark is reached on the graduated cylinder. Calibration of the Blot Washer occurs automatically.

7) Hold the 100 ml graduated cylinder while it fills at a level as close as possible to the level of the membrane boxes when operating the Blot Washer for automating Western Blot processing.

Setting the Aspiration Vacuum Factor

1) Press ◀ once if TO CALIBRATE DISPENSE 100 ML is displayed or press ◀ twice if SELECT NUMBER OF BLOTS is displayed.

```
SET ASPIRATION
VACUUM FACTOR: 5
```

Two elements affect the aspiration of liquids to waste: strength of vacuum and rate of shaker agitation on which the blot containers rest. If some liquid remains in the container after aspiration ceases, increase the numerical vacuum factor (maximum: 9). If aspiration continues long after all the liquid has been evacuated, decrease the vacuum factor (minimum: 1).

2) Use the ▲ or ▼ key to change the aspiration vacuum factor.

Chapter 4: Appendix

Accessory Part Numbers

ACCBW0002	4L Wash Bottle
ACCBW0003	140 ml Lock Syringe
ACCBW0005	Quad Pumping Harness
ACCBW0001	Collet w/Delivery Tube

Three different sizes of Stovall Western Incubation Boxes are available in various pack sizes. See www.slscience.com.

Specifications

Console Dimensions: 23 W x 9.6 D x 5.4 cm H (9″ W x 5.9″ D x 5.4″H).

Electrical:

115V model (BLWAA115S): 50-60 hz, 500 mAmp fuse.
220V model (BLWAA220S): 50-60 hz, 250 mAmp fuse.

Wash Duration: seconds to 99 hours per cycle.

Liquid Dispensing Rate: Approximately 2 ml/sec.

Liquid Aspiration Rate: Approximately 5 ml/sec.

Program Storage Capacity: 20 programs with up to 4 steps per program.

Liquid Flow Control Valves: 2 for delivery, 1 for aspiration.

CE

EC Declaration of Conformity

Stovall Life Science, Inc.
206 G. So. Westgate Drive
Greensboro, North Carolina, 27407 USA

Declares under our sole responsibility that the

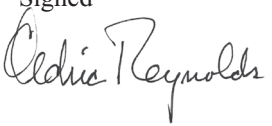
Blot Washer

is in conformity with the following EMC Standards:

EN50081-1: 1997	Generic emission standards for residential commercial, and light industry environments
EN50082-1: 1997	Generic immunity standards for residential commercial, and light industry environments

and therefore is in conformity with the
Protection Requirements of Council Directive 89/336/EEC
On the approximation of the laws of Member States
Relating to Electromagnetic Compatibility.

Signed



Date

12/29/03