Western Processor

Instruction Manual

Catalog Number
170-3970

BIO-RAD

Safety

Caution/Warning

Disconnect power to the Western Processor before servicing. No user-serviceable parts are inside the instrument. Refer servicing to Bio-Rad service personnel.

This instrument is intended for laboratory use only.

This product conforms to the “Class A” standards for electromagnetic emissions intended for laboratory equipment applications. it is possible that emmissions from this product may interfer with some sensitive applicances when placed nearby or in the same circuit as those applicances. The user should be aware of this potential and take appropriate measures to avoid interference.

The Western Processor is certified to meet the EN61010-1 safety standard for safety of laboratory equipment. Certified products are safe to use when operated in accordance with the instruction manual. This safety certification does not extend to other equipment or accessories not EN61010-1 certified, even when connected to the Western Processor.

This instrument should not be modified or altered in any way. Alteration of this instrument will void the manufacturer’s warranty, void the EN61010-1 certification, and create a potential safety hazard for the user.

Bio-Rad is not responsible for any injury or damage caused by the use of this instrument for purposes other than those for which it is intended or by modifications of the instrument not performed by Bio-Rad or an authorized agent.

EN61010-1 is an internationally accepted electrical safety standard for laboratory instruments.

Warranty

The Western Processor is warranted for one (1) year against defects in materials and workmanship. If any defects should occur during this warranty period, Bio-Rad Laboratories will replace the defective parts without charge. However, the following defects are specifically excluded:

1. Defects caused by improper operation.
2. Repair or modification done by anyone other than Bio-Rad Laboratories or their authorized agent.
3. Use with other spare parts not specified by Bio-Rad Laboratories.
4. Damage caused by deliberate or accidental misuse.
5. Damage due to use of improper solvent or sample including acetic acid and methanol.

For inquiry or request for repair service, contact your local Bio-Rad office.

Table of Contents

Section 1
Introduction

1.1 Overview

The Western Processor automates the western blot development process. After the antigen is applied to the membrane surface, the Western Processor will process the blot through the various washing, blocking, and incubation steps. Once the system is set-up with the required reagents and the appropriate assay is programmed, the Western Processor will proceed with the assay and alert you at certain critical steps and when the assay is completed.

The Western Processor is fully programmable from the front panel and can store up to ten (10) user defined assays. Each assay holds up to fifteen (15) steps where each step can contain up to six (6) cycles. In addition to these ten (10) user defined assays, the Western Processor contains five (5) pre-programmed assays designed specifically for use with Bio-Rad's immunoassay kits.

The Western Processor holds one, two, or four Mini-blot Trays for processing mini blots and one Standard Tray for processing up to 20 x 20 cm blots.

1.2 Features

The Western Processor provides the following features:

- Automatically controls incubation times, volumes, and washes.
- Six (6) programmable reagent delivery and manual reagent delivery cycles.
- Five (5) pre-programmed assays.
- Store up to ten (10) user-defined assays.
- Internal diaphragm pump aspirates reagents to waste bottle.
- The optional reagent rack holds six 50 ml tubes and two 1 liter bottles.
- By using the purge cycle, the tubing is easy to clean.
- Barbed fittings allow for easy removal and replacement of tubing.

1.3 Unpacking

When you receive the Western Processor, carefully inspect the shipping containers for any damage which may have occurred in shipping. Severe damage to a container may indicate damage to its contents. If you suspect damage to the contents may have occurred, immediately file a claim with the carrier in accordance with their instruction before contacting Bio-Rad Laboratories.

Open the shipping carton and lift the contents out of its packing. Check the contents of the box against the supplied packing list. Inspect the instrument for external damage. If any part is missing or damaged, contact Bio-Rad laboratories immediately.

Packing List

Western Processor
Standard Tray with lid, 1
Mini-blot Trays, 4
Waste bottle with lid and fittings
Dispensing and aspiration tubing assemblies including 3 tubing kits for 3 tray modes
External power supply with power cord
Tubing labels
Instructions

1.4 System Set-Up

1. Level the Western Processor on the work surface where you intend to operate. Connect the power cord to the external power supply and then plug the power supply into the back of the unit.
2. Turn the Western Processor on by pressing the ON/OFF power switch on the front panel of the unit.
3. The LCD displays the start-up screen with the software version, then the platform levels to the horizontal home position.
4. Unwrap the Standard Tray with lid, four Mini-blot Trays, and tubing kits.
5. Remove the dispensing and aspiration tube assemblies and reverse lid.
6. Place Standard Tray with lid on the platform.
7. Choose a tubing kit appropriate for the experiment (one tray, two tray or four tray), each of which has a dispensing tube assembly (smaller diameter tubing) and an aspiration assembly (straws on the end).
8. Connect the open fitting of the aspiration assembly to the tubing connected to the waste bottle cap. (When changing tubing kits for experiments with different numbers of trays, disconnect and reconnect from the same junction, the end of the tube coming from the waste bottle). Insert the straw end(s) into the 3 mm hole(s) above the tray positions you will be using as in the photographs in Figure 1.
9. Connect the tubing from the waste outlet on the back panel of the Western Processor to the second fitting in the waste bottle cap.
10. Connect the open fitting from the dispensing tube assembly to the 3mm tubing connected at the other end to the Y fitting after the pump. Put each dispensing end in the 0.8 mm hole in the lid next to each aspiration tube. Again refer to figure 1 for placement of the tubing kits for each tray configuration.

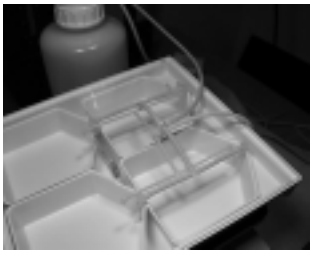


Fig. 1a. 4 tray configuration.

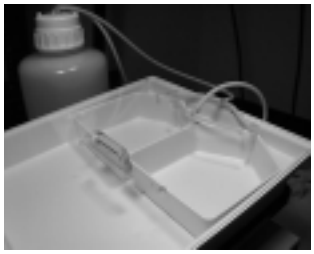


Fig. 1b. 2 tray configuration.

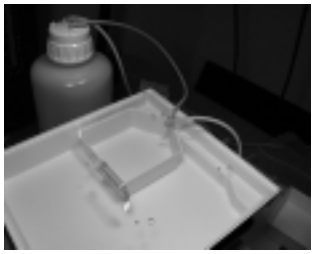


Fig. 1c. 1 tray configuration.

1.5 Description of System

Front Panel of Western Processor

ENTER: Use this key to select the message displayed on the LCD.

YES/», NO/«: These keys are used to scroll through the different options on the LCD and to select or decline messages displayed on the LCD.

SILENCE ALARM: This key is used to silence the alarm.

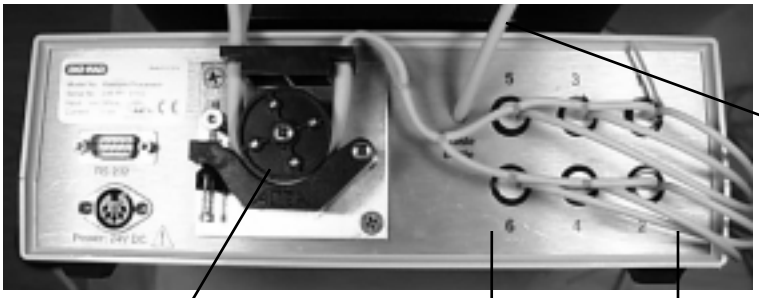
- Reagent preparation/addition alarm. When the program includes a step for manual addition of reagent, or the alarm is programmed in a step with automatic reagent addition, the alarm will sound at two different time points. The first alarm will sound five minutes prior to that step to alert the operator to prepare the required reagent. The second alarm sounds immediately prior to that step and prompts the operator to either prime the necessary valve, or add the reagent manually.
- Variable incubation alarm. Valve 4/SUBST allows for programming a variable incubation time. An alarm will sound at the end of the programmed time to alert the operator to monitor and stop the development.
- End of assay alarm. An alarm will sound after the final step of a assay.
- Manual aspiration alarm. When a "no aspiration" step is programmed, an alarm will sound to alert user to remove the reagent. This is generally used for a step where the blot was incubated with a valuable reagent that may be re-used. The platform continues rocking until the user reponds to the alarm. After the reagent is removed, the user is prompted to push a key to continue the assay.

PAUSE: During a run this key is used to temporarily stop the unit, this allows you to make changes during the run, or return to the run (page 5). During programming, this key can be used to access certain subroutines (page 6) or to escape to the beginning of a programming step, then to the beginning of a program.

ON/OFF: The Western Processor is turned on by pressing the ON/OFF switch on the front panel of the instrument.

The ON/OFF switch can also be used to reset the instrument. When resetting the instrument in the edit mode, all steps are saved to memory.

Rear panel of Western Processor



Section 2
System Operation

The Western Processor is designed to provide simple, intuitive screen layouts for programming. The system consists of four main modes that allow the operator to, I) run a programmed assay, II) edit or program an assay, III) calibrate the pump, IV) and purge tubing.

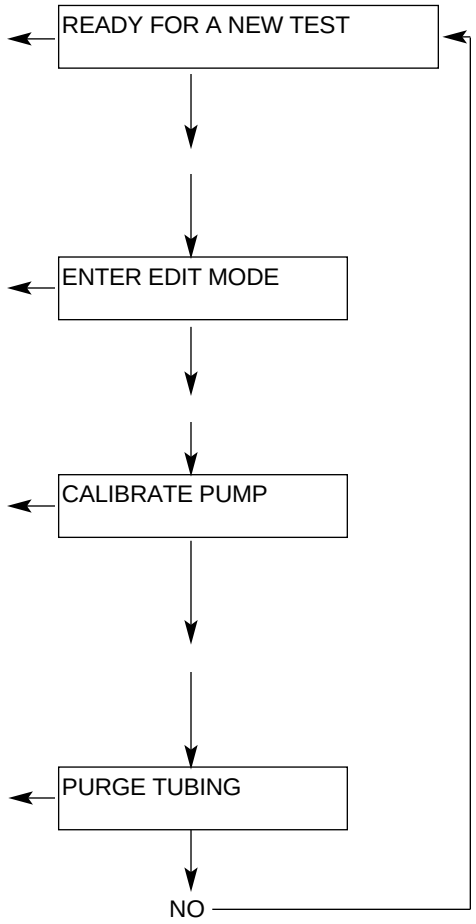
2.1 Flowchart of Main Operating Modes

Mode I: Running a Method
Start a programmed assay.

Mode II: Program/Edit a Method
Program a new method or edit a previously programmed method.

Mode III: Calibrate Pump Mode
Enter pump calibration mode. In this mode the instrument will adjust the "on" time of the pump to calibrate the dispensed volume The instrument comes factory-calibrated. Check the calibration periodically and whenever the pump tubing is replaced.

Mode IV: Purge Tubing Mode
Enter the manual or autoflush lines mode.



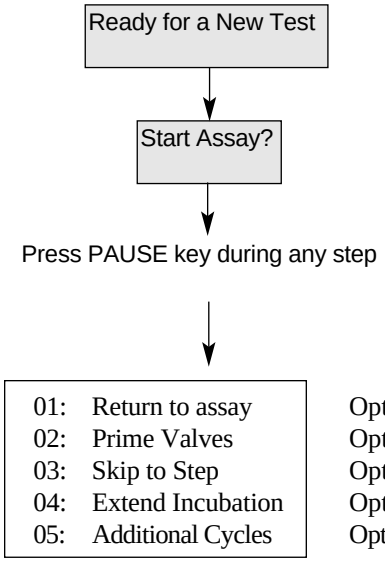
2.2 Flowcharts of Pause Subroutines

In addition to the four main operating modes, two subroutines are accessible with the PAUSE key.

PAUSE While Running a Method

This will let you make certain changes while running a method, such as priming valves, increasing incubation times, skipping a step, and adding additional cycles to a step. By putting the instrument in pause you have access to make these changes.

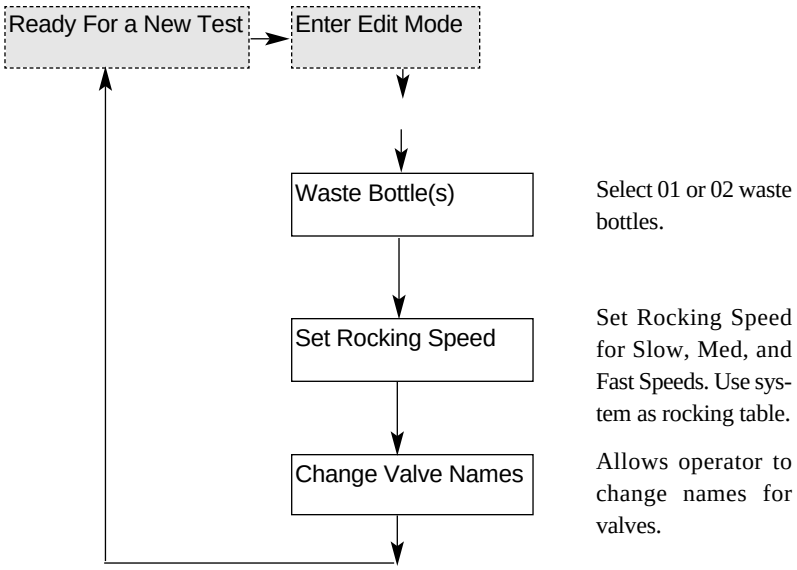
Note: Changes made while running a method are not permanent. When the method is completed, it reverts to the originally programmed parameters.



Option 01: Restarts assay at the step it was paused.
Option 02: Enters prime valve routine.
Option 03: Allows you to skip a step during a method.
Option 04: Increase the incubation of the paused step.
Option 05: Increase the number of cycles of the paused step.

PAUSE at the “Enter Edit Mode” Prompt

This will allow access to: 1) changing valve names, 2) changing the number of waste bottles (1 or 2), and 3) changing the rocking speed of the platform. In this mode you can also use the Western Processor as a simple rocking platform by turning the rocking motor on at a specified speed.



Note: The Set Rocking Speed will cycle through FAST, MED, and SLOW speed modes. The preset values for each of these rocking speeds are 30 RPM for FAST speed, 15 RPM for MED speed, and 5 RPM for SLOW speed.

Caution: All programmed methods will reflect the new valve names.

Section 3
Mode I–Running a Programmed Method

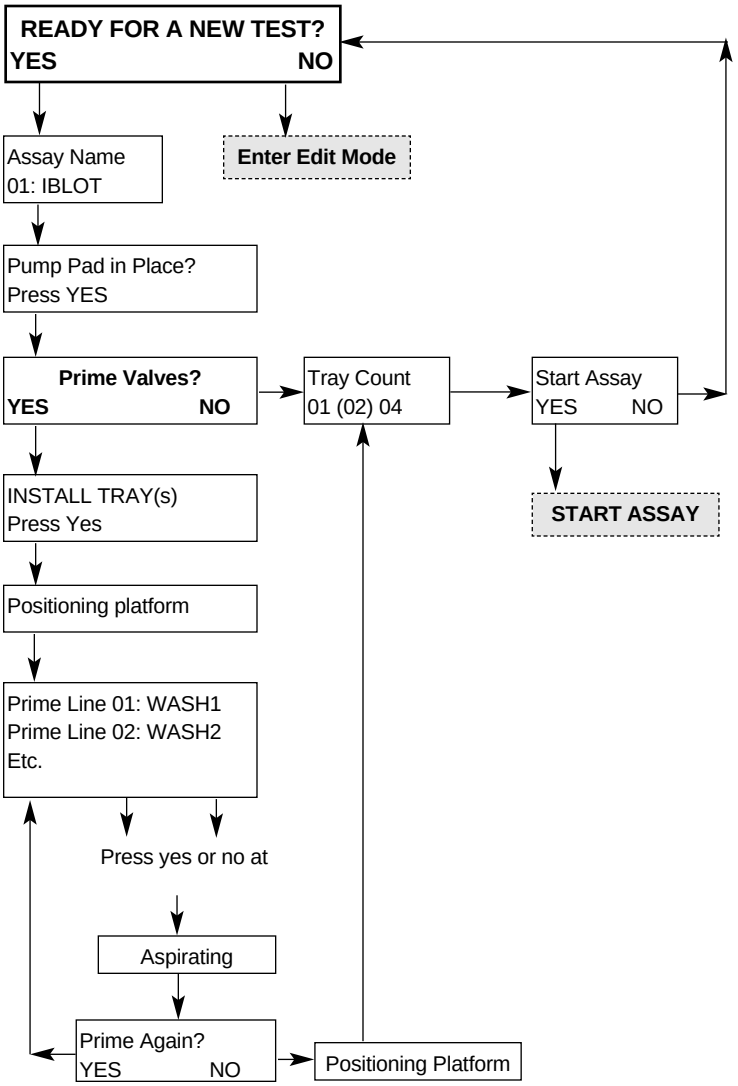
When the Western Processor is turned on, the prompt "Ready for a New Test" appears after the initial start up screen. By pressing the YES/» key, you enter a sequence of steps to start a programmed method.

The Western Processor contains five (5) pre-programmed assays to process mini-blots with Bio-Rad's immunoassay kits. These pre-programmed assays are designed to produce the optimum signal to noise ratio. The parameters of these pre-programmed assays cannot be changed. However, a similar assay can be programmed that is specifically tailored to your needs. For example, if you have a limited amount of primary antibody, you can reduce the primary antibody volume to as low as 5 ml. We recommend keeping the washing step volumes, rocking speeds, and times as listed in these assays. See Appendix B for details of the five assays.

The five pre-programmed assays and corresponding Bio-Rad immunoassay kit are:

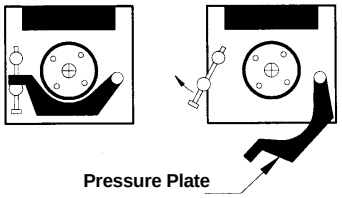
#1–IBLOT:	Bio-Rad Immun-Blot Kit
#2–AMPAP:	Bio-Rad Amplified Alkaline Phosphatase Immun-Blot Assay Kit
#3–OP4CN:	Bio-Rad Opti-4CN Detection Kit
#4–AOPTI:	Bio-Rad Amplified Opti-4CN Detection Kit
#5–ISTAR:	Bio-Rad Immun-Star Chemiluminescent Protein Detection Systems

3.1 Flowchart for Running a Programmed Method



Note: When a method is completed, be sure to immediately wash all six (6) lines and valves in the Purge Tubing Mode. If buffer salts and other reagents are allowed to remain in the system, the lifespan of the valves will be greatly reduced.

3.2 Sequence of Steps to Run a Programmed Method

Step #	LCD Display	Description
01	Ready for a New Test YES or NO	YES: Enters step sequence to start a method NO: Enters Edit Mode
02	Assay Name 01: ------	Use YES/» and NO/« keys to scroll through methods 01 to 15. Methods 1 through 5 are pre-programmed assays. Press ENTER to select the program displayed on the LCD
03	Pump Pad in Place PRESS YES	Make sure the pump pad on the back panel of the instrument is in place. Press YES to continue.
		 Pressure Plate
04	Prime Valves YES or NO	YES: Enters Prime Valves routine. This allows the operator to prime selected valves prior to starting a method. NO: Ready to start assay, go to step # 08 below
05	Install Tray(s) Press YES	Place tray(s) on the platform prior to priming the valves. One or two Mini-blot Trays, or one Standard Tray. Make sure the delivery- and aspirating- tubes are properly positioned in the lid. Press YES to continue. The platform will automatically position itself.
06	Prime Line 01: WASH1 Prime Line 02: WASH2 Etc.	YES: Primes that line NO: Advance to the next line. You can also prime the lines during the assay itself using the Pause Mode.
	YES or NO	Each valve will be sequentially displayed on the LCD. When all 6 lines are processed, the system will automatically aspirate the dispensed reagents.
07	Prime Again? YES or NO	YES: Repeat the priming sequence of step 06. NO: Ready to start assay. The platform will automatically position itself.
08	Tray Count 01 (02) 04	Use YES/» and NO/« keys to select 01, 02, or 04 trays. ENTER: Selects number displayed on LCD. (Selecting the number of trays will inform the Western Processor to deliver and aspirate the required volumes.)
09	Start Assay YES or NO	YES: Starts the selected Method Before pressing yes, make sure all the reagents are in place, and the necessary lines have been primed. Lines for reagents that are prepared just before use, can be primed in the pause mode during the assay. NO: Returns to “READY FOR A NEW TEST” mode.

Note: When a method is completed, be sure to immediately wash all six (6) lines and valves in the Purge Tubing Mode. If buffer salts and other reagents are allowed to remain in the system, the lifespan of the valves will be greatly reduced.

4.3 Sequence of Steps for Pause During Assay

While running a method the system can be paused to prime valves, to skip a step in the method, to extend the incubation time, or to add additional cycles to the paused step. To access these features, you can enter this subroutine any time during a method by pressing the PAUSE key.

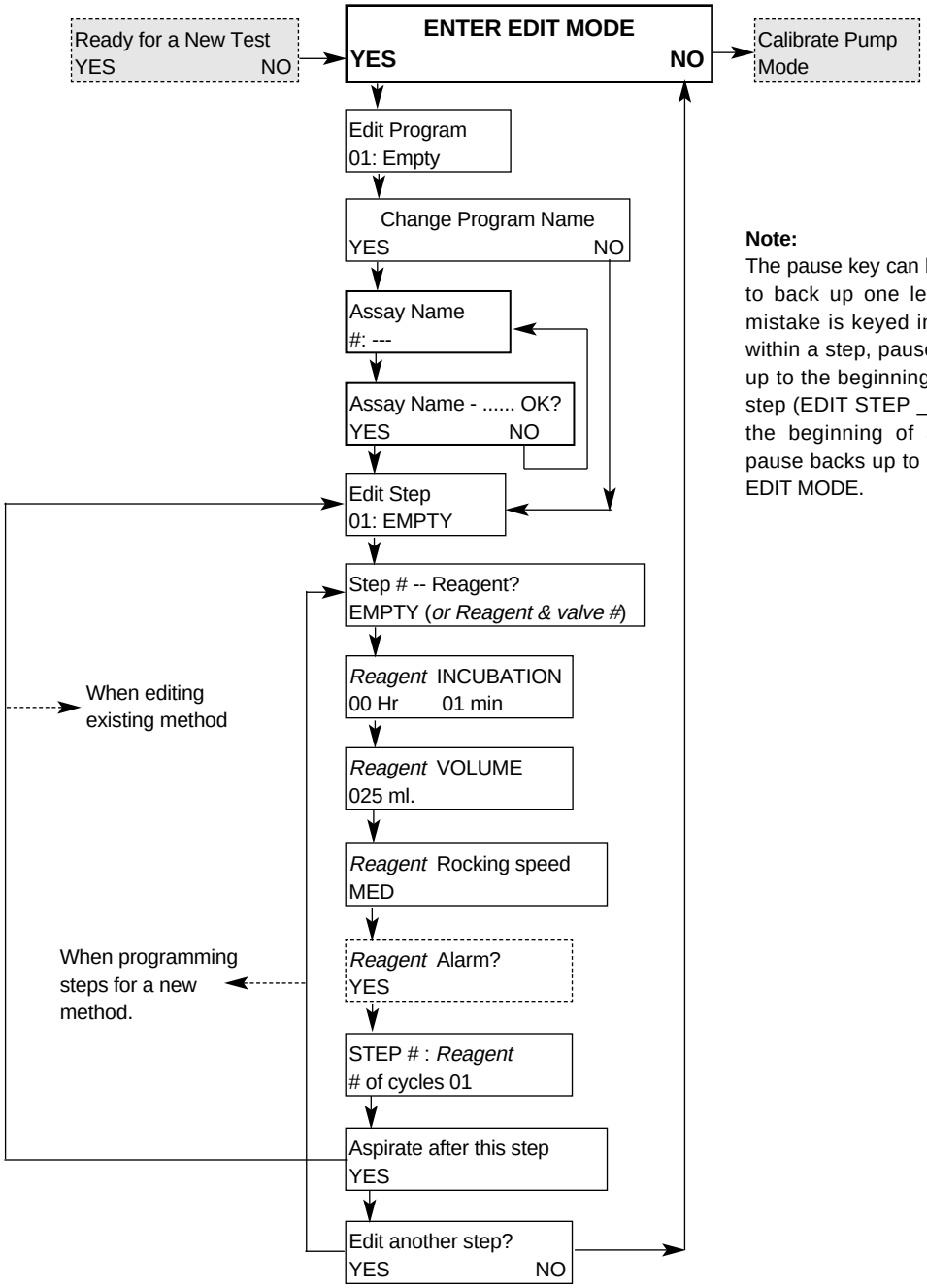
Note: Changes made while running a method are not permanent. When the method is completed, the method reverts to the originally programmed parameters.

Step #	LCD Display	Description
01	Step #: *Reagent* Cycle # *Time*	Press PAUSE to access special subroutine.
02	Pause Mode 01: Return to Assay	Use the YES/» and NO/« keys to scroll through the following five options: 01: Return to Assay 02: Prime Valves 03: Skip to Step 04: Extend Incubation 05: Additional Cycles. Press ENTER to select the option
03	Pause Mode 01: Return to assay	ENTER: Return to assay
04	Pause Mode 02: Prime Valves	ENTER: Enters the prime valve routine. Before starting the prime valve sequence, remove both dispense lines from the lid and place them in a container. Follow on screen prompts.
05	Pause mode 03: Skip to Step?	ENTER: Enters skip step routine.
05a	Skip to Step? #: Reagent	Use YES/» and NO/« keys to choose the step number. Press ENTER to select the step. Method proceeds with the selected step.
06	Pause Mode 04: Extend Incubation	ENTER: Enters incubation time routine. You can extend the incubation time of the paused step by sixty (60) minutes.
06a	Extend Incubation time by 01 min.	Use YES/» and NO/« keys to enter the time. The incubation time of the paused step will be extended by the time entered here. Press ENTER to select the entered time. Method proceeds where paused.
07	Pause Mode 05: Additional Cycles	ENTER: Enter additional cycles to routine
07a	Step#: *Reagent* Total Cycles 04	Use YES/» and NO/« keys to enter the number of cycles (01 to 06). The maximum number of cycles in a single step is six. Press ENTER to select the total number of added cycles. Method proceeds where paused.

Section 4
Mode II—Programming or Editing a Method

In this mode you can program a new method or edit an existing method. You can program up to 10 customized methods, each method consisting of up to 15 steps. Each step allows programming for reagent, incubation time, volume, rocking speed, and number of cycles. In addition, with subst/valve#4 reagent you can program a variable incubation time that alerts the operator to monitor the development step after a specified time.

4.1 Flowchart for Programming or Editing a Method



Note:
The pause key can be used to back up one level if a mistake is keyed in. From within a step, pause backs up to the beginning of that step (EDIT STEP _). From the beginning of a step, pause backs up to ENTER EDIT MODE.

4.2 Sequence of Steps to Program or Edit a Method

Note: Pause key can be used during programming to back up.

Step #	LCD Display	Description
01	Enter Edit Mode YES or NO	YES: Enters programming or edit mode, go to step 02 NO: Enters Pump Calibration Mode
02	Edit Program 01: Empty	Use the YES/» and NO/« keys to scroll through the list of programmed methods. Press ENTER to select the program displayed on the LCD screen. The program name "EMPTY" indicates a new method can be programmed. Selecting a previously programmed method allows you to edit any or all steps in that method. Note: Programs 1 through 5 are pre-programmed assays. No changes can be made to these assays. See Appendix B for details of these five assays.
03	Change Program Name YES or NO	YES: Name a new program or change the name of a previously programmed method. NO: Retain the displayed name, go to step # 6.
04	Assay Name # : —	Use the YES/» and NO/« keys to scroll through the alphabet and numbers. 012345689ABC….XYZ. The LCD screen displays a cursor under the space for the first character. Scroll to choose the selected character then press ENTER to select the character. The cursor moves to the next space for the following character. Each name can consist of up to 5 characters.
05	Assay Name ——-OK? YES or NO	YES: Saves displayed method name. NO: Rejects displayed method name, returns to step 04 to re-enter the name.
06	Edit Step 01: EMPTY When editing a method, the display will show the actual reagent name in place of EMPTY.	Press ENTER when programming a new method to enter step 1 of the method. When editing a method, use the YES/» and NO/« keys to scroll through the different steps, then press ENTER to select the step you wish to edit.
07	STEP # REAGENT? EMPTY (*or Reagent and valve#)*	Use the YES/» and NO/« keys to scroll through the different choices and press ENTER to accept the reagent/valve# choice. WASH1 Valve 1 WASH2 Valve 2 CONJ1 Valve 3 SUBST Valve 4 (allows for variable incubation time) CONJ2 Valve 5 REAGT Valve 6 MANUL (Allows for manual addition of reagent) SKIP Can be used to hide a step while editing a method END Returns to Enter Edit Mode prompt.

Step #	LCD Display	Description
08a	*Reagent* INCUBATION 00 HR 01 MIN (When editing, the display will show the programmed reagent name)	Use the YES/» and NO/« keys to enter the number of hours for the reagent incubation. Press ENTER to select the entered number of hours. Then use the YES/» and NO/« keys to enter the number of minutes for reagent incubation. Press ENTER to select the entered number of minutes.
08b	Variable Incubation YES	Use the YES/» and NO/« keys to choose YES or NO, then press ENTER to select the choice. Variable incubation is available only when SUBST/Valve 4 is selected. When variable incubation is selected, an alarm will sound at the end of the programmed incubation time. Incubation will continue until you press NO at the prompt. This allows you to monitor band development and stop the reaction when the bands are sufficiently developed.
9	*Reagent* VOLUME 025 ml	Use the YES/» and NO/« keys to enter the volume of reagents to be dispensed, from 000 ml to 500 ml in 1 ml increments. Press ENTER to select the number entered. **The volume entered is per tray**. When starting a method, you will be prompted to enter the number of trays used. The instrument will automatically dispense the programmed volume in each tray. When entering a MANUL step where reagent is manually added, you are prompted to enter the volume of the reagent you will add. (See Step 14)
10	*Reagent* Rocking Speed MED	Use the YES/» and NO/« keys to scroll through the Rocking Speed options. The Rocking Speed options are, ZERO, SLOW (5 rpm), MED (15 rpm), and FAST (30 rpm). These are the pre-set values. We recommend Medium–Slow Speed for incubation steps and Fast speed for wash steps. To change the pre-set values enter PAUSE subroutine in Enter Edit Mode.

Step #	LCD Display	Description
11	Reagent Alarm? YES	Use the YES/» and NO/« keys to choose Alarm, then press ENTER to select. If the step you define requires freshly prepared reagent, you can set the alarm to prompt you to prepare the reagent. When the alarm is selected, the alarm will first sound five minutes before the step that requires the freshly prepared reagent. A second alarm will sound when it is time to add the reagent. The instrument display will prompt you to prime the valve.
12	Step # *Reagent* # of cycles 01	Use the YES/» and NO/« keys to choose 01 to 06 cycles in that step, then press ENTER to select.
13A	Aspirate After This Step? YES	Use the YES/» and NO/« keys to select or deselect aspirate, then press ENTER to select. When a single cycle is selected in a step you can select to aspirate that step. If NO is selected, the instrument will not aspirate the reagent and the reagent for the next step be added to the tray(s). When multiple cycles are selected the instrument will automatically aspirate after each cycle except when it is the final step in a method. In that case the last cycle of that step will not be aspirated to prevent drying out of the blot(s).
13B		*If two (2) waste bottles were selected in the Edit Mode Pause subroutine, you will now be asked to assign waste bottle 01 or 02 with each step. If waste bottle 02 is selected an alarm will sound upon completion of that step to manually transfer the waste lines to the second waste bottle, or manually remove reagent. For example, to recover primary antibody, selecting waste bottle 2 allows you to recover the primary antibody.*
14	MANUL Asp Volume? 25 ml	This display appears only when you have selected MANUL for reagent. Entering the volume of the reagent you will manually add per tray alerts the instrument to the volume to be aspirated. Use the YES/» and NO/« keys to choose volume (000 to 500ml) then press ENTER to select. Reagent can be recovered by using the 2 waste bottle option.
15	Edit Another Step? YES or NO	YES: Return to Step 7 in this programming sequence to program another step. NO: Return to “ENTER EDIT MODE” screen.

4.3 Sequence of Steps for Pause in Edit Mode

A separate subroutine can be accessed through the ENTER EDIT MODE screen. This subroutine allows the operator to set the number of waste bottles, to set the rocking speed, and to change the valve names.

Step #	LCD Display	Description
01	Enter Edit Mode YES NO	Press PAUSE to enter subroutine
02	Waste Bottle(s) 01	Use YES/» and NO/« keys to enter 01 or 02 waste bottles. When one waste bottle is selected all reagents will be aspirated into this single container. Select two waste bottles if you wish to retain certain reagents. When two waste bottles are selected, you will be asked at each step during programming whether the reagent will be aspirated in waste bottle 1 or 2.
03	Set Rocking Speed YES NO	YES: Enters Set "Fast Rocking Speed:" NO: Enters Change Valve Names–go to step 8
04	Set Fast Rocking? YES NO	YES: Enter screen to set Fast Rocking Speed. NO: Enters Set Med Rocking Speed.
05	Fast Rock RPM=030	Use YES/» and NO/« keys to scroll to the desired rocking speed (05 to 035 RPM). Press ENTER to select the desired speed.
06	Run Rock Motor YES NO	YES: Starts rocking motor at set RPM. NO: Return to Set Fast Rocking screen-go to step 4
07	Rock Motor On Press NO to stop	This mode lets you use the Western Processor as a standard rocking platform. Press NO to stop the motor and return to "Set Fast Rocking" screen
	Note: The Set Rocking Speed will cycle through FAST, MED, and SLOW speed modes. The preset values for each of these rocking speeds are 030 RPM for FAST speed, 015 RPM for MED speed, and 005 RPM for SLOW speed.	
08	Change Valve Names YES NO	YES: Enters Changing Valve Name routine NO: Returns to "Enter Edit Mode" screen
09	Valve 01: WASH1 Change? YES NO	YES: Allows user to change name of Valve 01 NO: Advances to Valve 02
10	Valve Name 01: ——-	Use YES/» and NO/« keys to scroll through alphabet and numbers. The name can consist of up to 5 characters. After entering each character press ENTER to select that character and advance to the next character. Returns to "Enter Edit Mode" screen when completed.

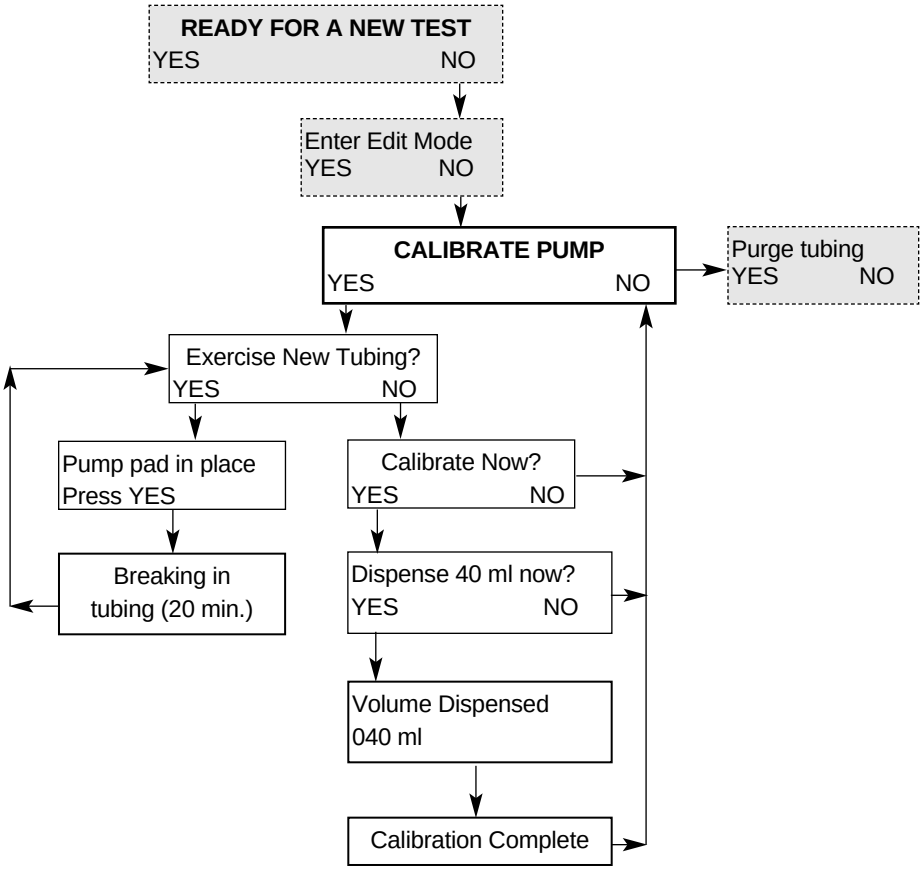
Note: The routine will scroll through all six valves. You can rename any of the valves. The default valve names are: #1 WASH1, #2 WASH2, #3 CONJ1, #4 SUBST, #5 CONJ2, #6 REAGT.

Caution: All programmed methods will reflect the new valve names.

Section 5
Mode III—Calibrate Pump Mode

Pump calibration adjusts the time necessary for the pump to deliver a certain volume. The Pump Calibration mode is accessed by pressing the NO/« keypad at the "ENTER EDIT MODE?" prompt. The instrument comes factory calibrated. However, calibration should be checked for accuracy periodically and when the pump tubing is replaced. If the dispensed volume is incorrect, check that the tubing and nozzles are clean and in good condition. To calibrate the dispensed volume, use the Pump Calibration Routine below.

5.1 Flowchart for CALIBRATE PUMP MODE



5.2 Sequence of Steps to Calibrate Pump

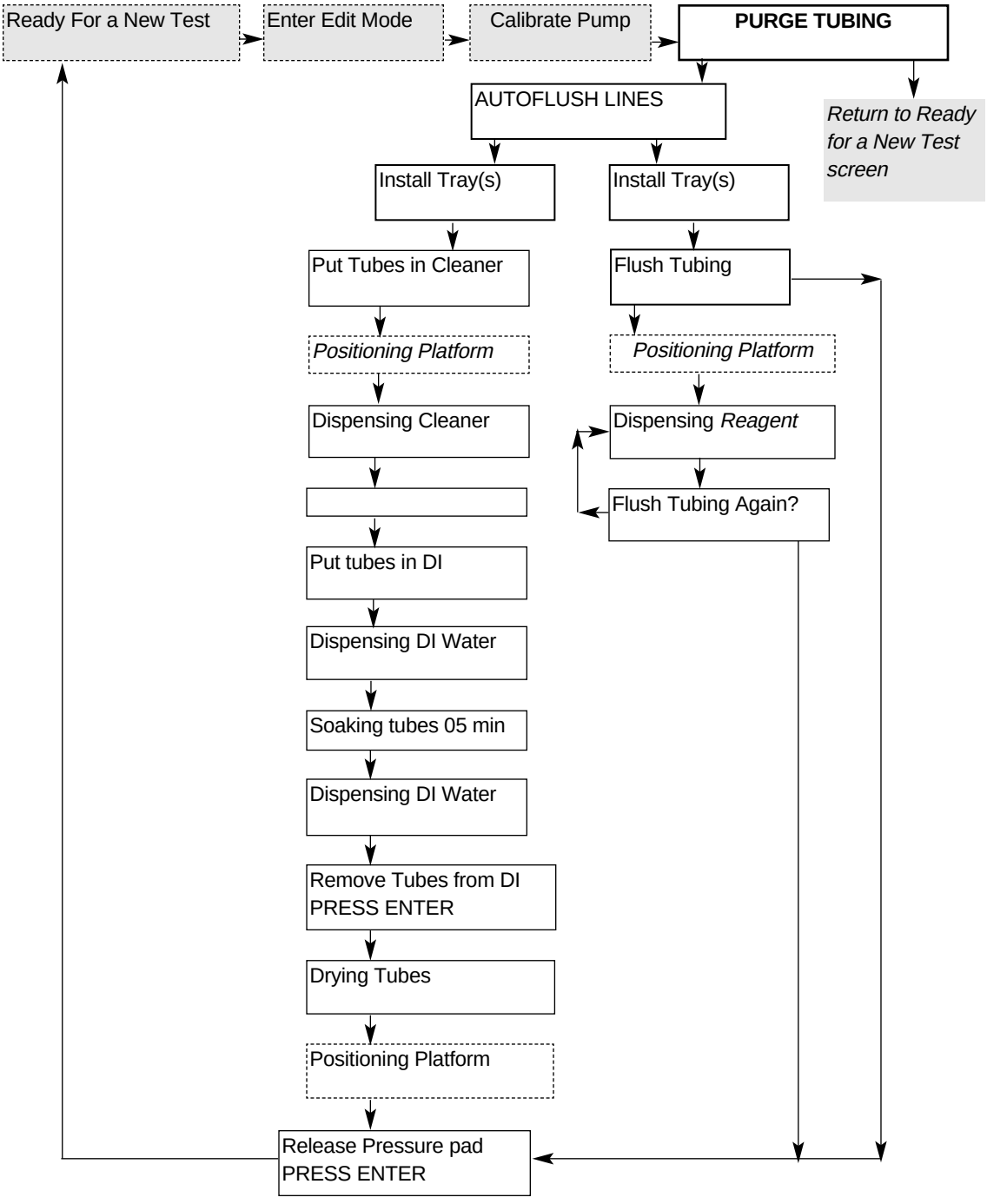
Before calibrating the pump, make sure the pressure pad on the rear panel of the instrument has been locked into place.

Step #	LCD Display	Description
01	Calibrate Pump? YES NO	YES: Enter Pump Calibration Routine NO: Enter Purge Tubing Routine
02	Exercise New Tubing? YES NO	YES: Exercises new tubing (~20 minutes). LCD Display will prompt you to make sure the pressure pad is in place. The tubing supplied with the instrument does not require the exercise routine. This routine is used for new tubing. DO NOT use any fluid in the system during the exercise routine. NO Enter Pump Calibration (skip exercise tubing routine).
03	Calibrate Now? YES NO	Before calibrating the pump make sure the pressure pad has been locked into place for at least one hour in order to approximate real-life operating conditions. It is also best to prime the pump before starting calibration. YES: Continue Calibrate Pump Routine. NO: Return to Calibrate Pump Mode.
04:	Dispense 40 ml Now? YES NO	Remove dispense tube from dispense tubing at fitting juction and place in a 50 ml graduated cylinder. Make sure the valve 1 feed line is placed in water before pressing YES. YES: The pump will start and deliver 40 ml. NO: Return to Calibrate Pump Routine
05:	Volume Dispensed 40 ml	ENTER : Volume delivered is 40 ml, return to Calibrate Pump Mode. If volume delivered is other than 40 ml, use the YES/» and NO/« keys to enter the actual volume delivered, then press ENTER. Return to step 03, Calibrate Now, to verify calibration.

Section 6
Mode IV—Purge Tubing Mode

The tubing and valves should be purged at the completion of each assay to insure the lines are free of contamination before the next assay. To remove any latent bacteria we recommend using a 2% bleach solution or a commercially available cleaning preparation that will not interfere with your assay(s) or damage the tubing.

6.1 Flowchart for Purge Tubing Mode



6.2 Sequence of Steps to Purge Tubing

For optimum performance we recommend utilizing the purge tubing routine after each assay. During the Purge Tubing Mode, the tubing from each valve is cleaned. The Purge Tubing Mode is accessed via the Pump Calibration routine or at the completion of an assay. Below are the step by step detailed instructions for purging the tubing.

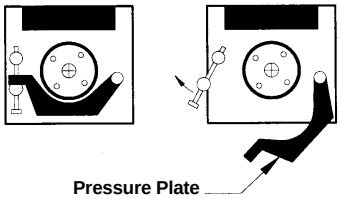
Step #	LCD Display	Description
01	Purge Tubing? YES NO	YES: Enter Purge Tubing Routine NO: Return to Ready for New Test
02	Autoflush Tubes? YES NO	YES: Enters sequence of three flushing cycles, 1) wash and soak, 2) rinse and soak, and 3) dry. GO TO STEP 03 then 06. NO: Enters sequence of steps for single wash cycles. FOLLOW STEPS 03 and 04.
03	Install Tray(s) Press YES	Make sure a tray is on the platform, then press YES. Make sure the two dispense lines and the two aspirate lines are positioned over the tray(s). Make sure the six tubes are placed in the appropriate wash solution.
04 *Manual Flush Mode*	Flush Tubing? YES NO	YES: Enter manual flush tubes routine. Platform positions itself automatically. NO: Return to Purge Tubing screen.
05	Dispensing Valve 1, 2, 3,....6	The instrument will sequentially wash each of the six tubes. After completion, the dispensed solution will be aspirated and the routine enters "Flush Tubing Again" to repeat washing/rinsing cycle or to return to Purge Tubing Screen.
06 *Autoflush Mode*	Put Tubes in Cleaner Press ENTER	Place the six tubes in the appropriate cleaning solution, then press ENTER.
07	Dispensing Cleaner Valve 1, 2, 3,....6	The instrument will sequentially pump cleaning solution through each of the six tubes. After completion the dispensed solution will be aspirated.
08	Soaking Tubes 05 min	The tubes will soak for 05 minutes in the cleaning solution. After 5 minutes the alarm will sound.
09	Put tubes in DI Press ENTER	Place the six tubes in a container with deionized water and press ENTER.
10	Dispensing DI water Valve 1, 2, 3,....6	The instrument will sequentially pump deionized water through each of the six tubes. After completion the dispensed solution will be aspirated.
11	Soaking Tubes 05 min	The tubes will soak for 05 minutes in di water. A second wash will automatically start, step 12.
12	Dispensing DI water Valve 1, 2, 3,....6	For the second wash, the instrument will sequentially pump deionized water through each of the six tubes. After completion the dispensed solution will be aspirated. The alarm will sound to alert you to step 13.

Step #	LCD Display	Description
13	Remove tubes from DI. Press ENTER	Remove the tubes from the di water. ENTER: Starts pump to remove remaining deionized water and air flush the tubes.
14	Drying tubes 05 min. Valve 1, 2, 3,....6	
15	Positioning Platform	Platform will automatically position itself.
16	Release Pressure Pad Press ENTER	Unlock the pump pressure pad from the pump on the rear panel of the instrument. ENTER: Returns to Ready for a New Test screen.

Section 7
System Maintenance

7.1 Pump Pressure Plate

The spring loaded pressure plate on the peristaltic pump should be unlocked when the instrument is not in use to minimize wear on the tubing.



The tension setting on the pump release is preset for optimum performance. Changing the tension of the pump release will adversely affect the calibration and the lifespan of the tubing.

7.2 Valve and Tube Cleaning

The valves and tubing should be kept clean to ensure good pumping action. Upon completion of an assay the operator is given the opportunity to purge the tubing. You can choose to purge the tubing in the manual or automatic mode. See Section 6 for detailed steps on the Purge Tubing Mode. To clean the tubing, we recommend you use a 2% bleach solution or a commercially available preparation to remove any latent bacteria. In the automatic purge tubing mode, the lines are soaked for five minutes with the cleaning solution. Subsequent washes remove the cleaning solution and dissolve any accumulation of salt.

7.3 Tube Replacement

Tube replacement kits are available, see Appendix C for product ordering information. The tubing in these kits is cut to the proper length. Proper tube length and position are critical for proper performance of the peristaltic pump. The pump tubing has an approximate life of 1,000 hours of pumping. Pumping occurs for a fraction of assay time and is dependent on volumes delivered. If you notice liquid on the bench - change the pump tubing. It is a good idea to have a set of replacement pump tubing available in your lab.

Appendix A
Troubleshooting

Problem	Cause	Solution
Reagents are not dispensed	1. Tubing is not connected.	1. Connect tubing.
	2. Reagent bottle(s) are empty.	2. Make sure appropriate reagent bottles are filled.
	3. Pump Pressure Pad is in unlocked position	3. Place Pump Pressure Pad in locked position.
LCD display does not light up when instrument is turned on	Instrument not plugged into the power pack and/or wall outlet	Make sure the instrument is plugged into the power supply and into the electrical outlet.
Splashing during dispense cycle	Tubes and/or nozzles are plugged	Clean or change tubing and nozzle. If tubing is changed proceed through the exercise tubing and pump calibration routine.
"Rock Motor Steps Lost" display error	Rock Motor fails to rock platform correctly.	1. If the platform is still rocking. Make sure there is nothing impeding the movement of the rocker mechanism.
		2. If the platform is no longer rocking. Call Bio-Rad to return instrument for service.
Instrument fails to aspirate dispensed reagents.	1. Waste bottle not sealed.	1. Make sure the waste bottle fittings are in place and the lid is secure.
	2. Aspiration pump not working properly.	2. If the aspiration pump is not functioning call Bio-Rad to return instrument for service.
	3. No vacuum.	3. Check for vacuum at the hose extending from the back of the instrument. If vacuum is present at the hose, change out tubing form this point to the bottle and from the bottle to the aspiration tip.

Problem	Cause	Solution
Unsatisfactory results. *E.g.* high background.	1. Ran incorrect assay.	1. Make sure the correct assay is selected and programmed.
	2. Washing steps missing or inadequate.	2. Increase the number of cycles, volume, or rocking speed of the wash steps.
	3. Inactive reagents	3. Check the manual for the assay used to check the reagents used.

Appendix B

The Western Processor contains five (5) pre-programmed assays to process mini-blots. The parameters for each assay are listed below. These pre-programmed assays can be used as templates for programming new assays to process large blots. Simply increase incubation volumes to 50 ml and wash volumes to 100–200 ml. Large blots may require a larger waste bottle, which can be obtained from Nalgene Inc. (Heavy Duty Vacuum Bottle, catalog number 2126-4000, and Filling/Venting Closure, catalog number 2162-0831)

Assay # 1: IBLOT

The Immun-Blot assay kits are enzyme immunoassays kits optimized for the detection of specific antigens. The kits provide all necessary components and chemicals. This pre-programmed assay is designed to produce the optimum signal to noise ratio. The parameters listed for each step cannot be changed in this pre-programmed assay. However, a similar assay can be programmed that is specifically tailored to your needs. For example, if you have a limited amount of primary antibody, you can reduce the primary antibody volume in step 4 to as low as 5 ml. We do recommend keeping the washing step volumes, rocking speeds, and times as listed in this assay.

Note: When using the Protein G-HRP, the unbound primary antibody is removed with TCBS pH 5.5, rather than TTBS pH 7.5. The optimal binding pH of Protein G is 5.5. This assay can be used by manually exchanging the buffer at step 5, or by programming a new assay.

Reagents Required

Valve #1 - WASH1: TTBS, Tris buffered saline, 20 mM Tris, 500 mM NaCl, 0.05% Tween-20, pH 7.5

Valve #2 - WASH2: DI water

Valve #3 - CONJ1: Primary antibody

Valve #4 - SUBST: Alkaline phosphatase- or horseradish peroxidase color development reagents

Valve #5 - CONJ2: Secondary antibody conjugate

Valve #6 - REAGT: Blocking solution

Step #	Valve #/ Name	Reagent	# Cycles/ Time	Volume/ Speed	Alarm/Aspirate/ Variable Incubation
1	1: WASH1	TTBS	1 x 5 min	40 ml/Fast	No/Yes/NA
2	6: REAGT	Blocking solution	1 x 60 min	20 ml/Med	No/Yes/NA
3	1: WASH1	TTBS	3 x 5 min	40 ml/Fast	No/Yes/NA
4	3: CONJ1	Primary antibody	1 x 60 min	20 ml/Med	No/Yes/NA
5	1: WASH1	TTBS	3 x 5 min	40 ml/Fast	No/Yes/NA
6	5: CONJ2	Secondary antibody	1 x 60 min	20 ml/Med	No/Yes/NA
7	1: WASH1	TTBS	4 x 5 min.	40 ml/Fast	No/Yes/NA
8	4: SUBST	developer	> 10 min	20 ml/Med	No/Yes/Yes
9	2: WASH2	diH_2O	2 x 5 min	40 ml/Fast	No/No/NA
10	End				

Note: In step 8 an alarm will sound after 10 minutes incubation with substrate. At this point, you can elect to stop or continue the development process.

Assay # 2: AMPAP

The Amplified Alkaline Phosphatase Immun-Blot assay kit is an enzyme immunoassay system utilizing biotin-streptavidin for increased sensitivity in detection of specific antigens. The kit provides all necessary components and chemicals. This pre-programmed assay is designed to produce the optimum signal to noise ratio. The parameters listed for each step cannot be changed in this pre-programmed assay. However, a similar assay can be programmed that is specifically tailored to your needs. For example, if you have a limited amount of primary antibody, you can reduce the primary antibody volume in step 4 to as low as 5 ml. We do recommend keeping the washing step volumes, rocking speed, and times as listed in this assay.

Reagents Required

Valve #1 - WASH1: TTBS, Tris buffered saline, 20 mM Tris, 500 mM NaCl, 0.05% Tween-20, pH 7.5

Valve #2 - WASH2: DI water

Valve #3 - CONJ1: Primary antibody

Valve #4 - SUBST: Alkaline Phosphatase color development reagents

Valve #5 - CONJ2: Secondary antibody - alkaline phosphatase conjugate.

Valve #6 - REAGT: Blocking solution

MANUL: Streptavidin-biotinylated alkaline phosphatase complex. When preparing this reagent allow the complex to incubate for 1–3 hours before using. If allowed to form over 3 hours, remake the solution. See manual for details.

Step #	Valve #/ Name	Reagent	# Cycles/ Time	Volume/ Speed	Alarm/Aspirate/ Variable Incubation
1	1: WASH1	TTBS	2 x 5 min	40 ml/Fast	No/Yes/NA
2	6: REAGT	Blocking solution	1 x 60 min	20 ml/Med	No/Yes/NA
3	1: WASH1	TTBS	3 x 5 min	40 ml/Fast	No/Yes/NA
4	3: CONJ1	Primary Ab	1 x 60 min	20 ml/Med	No/Yes/NA
5	1: WASH1	TTBS	3 x 5 min	40 ml/Fast	No/Yes/NA
6	5: CONJ2	Secondary Ab	1 x 60 min	20 ml/Med	No/Yes/NA
7	1: WASH1	TTBS	3 x 5 min	40 ml/Fast	No/Yes/NA
8	MANUL	Streptavidin-AP	1 x 60 min	20 ml/Med	Yes/Yes/NA
9	1: WASH1	TTBS	4 x 5 min	40 ml/Fast	No/Yes/NA
10	4: SUBST	developer	≥ 10 min	20 ml/Med	No/Yes/Yes
11	2: WASH2	diH_2O	2 x 5 min	40 ml/Fast	No/No/NA
12	End				

Note: In step 10 an alarm will sound after 10 minutes incubation with substrate. At this point you can elect to stop or continue the development process.

Assay # 3: OP4CN

The Opti-4CN substrate kit is a more sensitive version of the colorimetric horseradish peroxidase (HRP) Immun-Blot kit. The substrate, 4-chloro-1-naphthol (4CN) is replaced with Opti-4 CN substrate producing a 4–8 fold increase in detection sensitivity. The parameters listed for each step cannot be changed in this pre-programmed assay. However, a similar assay can be programmed that is specifically tailored to your needs. For example, if you have a limited amount of primary antibody, you can reduce the primary antibody volume in step 4 to as low as 5 ml. We do recommend keeping the washing step volumes, rocking speed, and times as listed in this assay.

Reagents Required (See manual for details of reagent preparation)

Valve # 1 - WASH1: PBST, Phosphate buffered saline / 0.1% Tween-20

Valve # 2 - WASH2: DI water

Valve # 3 - CONJ1: Primary antibody

Valve # 4 - SUBST: Opti-4 CN substrate

Valve # 5 - CONJ2: Secondary antibody-HRP conjugate

Valve # 6 - REAGT: Blocking solution

Step #	Valve #/ Name	Reagent	# Cycles/ Time	Volume/ Speed	Alarm/Aspirate/ Variable Incubation
1	1: WASH1	PBST	2 x 5 min	40 ml/Fast	No/Yes/NA
2	6: REAGT	Blocking solution	1 x 60 min	20 ml/Med	No/Yes/NA
3	1: WASH1	PBST	3 x 5 min	40 ml/Fast	No/Yes/NA
4	3: CONJ1	Primary Ab	1 x 60 min	20 ml/Med	No/Yes/NA
5	1: WASH1	PBST	3 x 5 min	40 ml/Fast	No/Yes/NA
6	5: CONJ2	Secondary Ab	1 x 60 min	20 ml/Med	No/Yes/NA
7	1: WASH1	PBST	4 x 5 min	40 ml/Fast	No/Yes/NA
8	4: SUBST	Opti-4CN Substrate	≥ 10 min	20 ml/Med	Yes/Yes/Yes
9	2: WASH2	diH_2O	2 x 5 min	40 ml/Fast	No/No/NA
10	End				

Note: In step 8 an alarm will sound after 10 minutes incubation with substrate. At this point, you can elect to stop or continue the development process.

Assay # 4: AOPTI

The Amplified Opti-4 CN enzyme immunoassay kit results in an additional 4–8 fold signal amplification compared to the Opti-4 CN enzyme immunoassay kit. In addition to replacing the 4-chloro-1-naphthol (4CN) substrate with Opti-4 CN substrate the amplified Opti-4 CN kit uses streptavidin-HRP to further amplify the signal. The parameters listed for each step cannot be changed in this pre-programmed assay. However, a similar assay can be programmed that is specifically tailored to your needs. For example, if you have a limited amount of primary antibody, you can reduce the primary antibody volume in step 4 to as low as 5 ml. We do recommend keeping the washing step volumes, rocking speed, and times as listed in this assay.

Reagents Required (See manual for details of reagent preparation)

Valve # 1 - WASH1: PBST, Phosphate buffered saline / 0.1% Tween-20

Valve # 2 - WASH2: 20% DMSO/PBST

Valve # 3 - CONJ1: Primary antibody

Valve # 4 - SUBST: Opti-4 CN substrate

Valve # 5 - CONJ2: Secondary antibody-HRP conjugate

Valve # 6 - REAGT: Blocking solution

Additional reagents for manual addition at steps 8 and 11:

Step 8 - MANUL: Bio-Rad Amplification Reagent (BAR), see manual for preparation

Step 11 - MANUL: Streptavidin-HRP reagent

Step #	Valve #/ Name	Reagent	# Cycles/ Time	Volume/ Speed	Alarm/Aspirate/ Variable Incubation
1	1: WASH1	PBST	2 x 5 min	40 ml/Fast	No/Yes/NA
2	6: REAGT	Blocking solution	1 x 60 min	20 ml/Med	No/Yes/NA
3	1: WASH1	PBST	3 x 5 min	40 ml/Fast	No/Yes/NA
4	3: CONJ1	Primary Ab	1 x 60 min	20 ml/Med	No/Yes/NA
5	1: WASH1	PBST	3 x 5 min	40 ml/Fast	No/Yes/NA
6	5: CONJ2	Secondary Ab	1 x 60 min	20 ml/Med	No/Yes/NA
7	1: WASH1	PBST	4 x 5 min	40 ml/Fast	No/Yes/NA
8	MANUL	BAR	1 x 10 min	20 ml/Med	Yes/Yes/Yes
9	2: WASH2	20% DMSO/PBST	4 x 5 min	40 ml/Fast	No/Yes/NA
10	1: WASH1	PBST	3 x 5 min	40 ml/Fast	No/Yes/NA
11	MANUL	Streptavidin-HRP	1 x 30 min	20 ml/Med	Yes/Yes/NA
12	1: WASH1	PBST	3 x 5 min	40 ml/Fast	No/Yes/NA
13	4: SUBST	Opti-4CN Substrate	≥ 10 min	20 ml/Med	Yes/Yes/Yes
14	End	Transfer manually to diH_2O to stop rxn			

Note: In step 13 an alarm will sound after 10 minutes incubation with substrate. At this point you can elect to stop or continue the development process.

Assay # 5: ISTAR

The Immun-Star chemiluminescent detection system is a non-isotopic method for immunodetection of specific antigens immobilized on nitrocellulose or PVDF membrane. This system uses the CDP-Star chemiluminescent substrate and enhancer which is activated by an alkaline phosphatase enzyme conjugate. The assay, ISTAR, processes the blot to the development step. At that point the blot is removed from the Western Processor and development is carried out as described in the manual. The parameters listed for each step cannot be changed in this pre-programmed assay. However, a similar assay can be programmed that is specifically tailored to your needs. For example, if you have a limited amount of primary antibody, you can reduce the primary antibody volume in step 4 to as low as 5 ml. We do recommend keeping the washing step volumes, rocking speed, and times as listed in this assay.

Reagents Required (See manual for details of reagent preparation)

Valve # 1 - WASH1: TTBS, Tris buffered saline/0.1% Tween-20

Valve # 2 - WASH2: TBS, Tris buffered saline

Valve # 3 - CONJ1: Primary antibody

Valve # 4 - CONJ2: Secondary antibody-HRP conjugate

Valve # 5 - REAGT: Blocking solution

Step #	Valve #/ Name	Reagent	# Cycles/ Time	Volume/ Speed	Alarm/Aspirate/ Variable Incubation
1	2: WASH2	TBS	2 x 5 min	40 ml/Fast	No/Yes/NA
2	5: REAGT	Blocking solution	1 x 60 min	20 ml/Med	No/Yes/NA
3	1: WASH1	TTBS	3 x 5 min	40 ml/Fast	No/Yes/NA
4	3: CONJ1	Primary Ab	1 x 60 min	20 ml/Med	No/Yes/NA
5	1: WASH1	TTBS	3 x 5 min	40 ml/Fast	No/Yes/NA
6	4: CONJ2	Secondary Ab	1 x 60 min	20 ml/Med	No/Yes/NA
7	1: WASH1	TTBS	4 x 5 min	40 ml/Fast	No/Yes/NA
8	End	Transfer manually to diH_2O to stop rxn			
	Remove membrane from wash and proceed manually with development.				

Development of the membrane is done manually on a piece of saran wrap. After 5 minutes it is placed in a bag or in saran wrap to prevent membrane from drying out. Depending on the type of membrane and developer used, the film is exposed anywhere from 1–10 minutes or 12–24 hours. See manual for details.

Appendix C
Product Information and Specifications

Catalog Number	Description
170-3970	**Western Processor, 100–240 VAC**, Western Processor unit with integral rocking platform, user interface, peristaltic and diaphragm pump. Also includes standard tray with clear plastic lid, waste bottle, four mini-blot trays , 3 aspiration and dispense tubing kits for one, two or four tray configurations, manual
170-3973	**Reagent Rack**, holds two 1-liter bottles of wash buffer and six reagent vials
170-3974	**Mini-blot Trays**, 10, 8.5 cm x 10 cm
170-3975	**Mini-blot Trays**, 50, 8.5 cm x 10 cm
170-3976	**Standard Trays**, 2, 21.5 cm x 21 cm
170-3972	**Acrylic Lid**, Standard Tray, 1
170-3978	**Waste Bottle**, includes lid and fittings
170-3980	**Tubing kit; semi complete**. Reagent through dispense connection, includes all tubing and connectors for complete replacement from reagent tubes, tubing connected to valves, through the dual channel pump, to the connection with aspiration and dispense kits (170-3982, 170-3983, and 170-3984)
170-3981	**Peristaltic Pump Tubing Kit**, includes four dual tubing assemblies with snap in pump fittings and through Y connectors on either end
170-3982	**4 Tray Aspiration and Dispense Kit**, includes four each of aspiration and dispense tubing and fittings through the connector to the tubing kit (170-3980). This kit connects to four holes in the tray lid for each dispense and aspiration into four mini trays
170-3983	**2 Tray Aspiration and Dispense Kit**, includes four each of aspiration and dispense tubing and fittings through the connector to the tubing kit (170-3980). This kit connects to two holes in the tray lid for each dispense and aspiration into two mini trays or large tray
170-3984	**1 Tray Aspiration and Dispense Kit**, includes four each of aspiration and dispense tubing and fittings through the connector to the tubing kit (170-3980). This kit connects to one hole in the tray lid for dispense and aspiration into one mini tray
100-1547	**Western Processor Service Manual**

Specifications

Parameter	Standard Tray	Mini-blot Tray
Number of trays	1	4
Capacity	40 to 200 + ml	8–50 ml
Blots per run*	1	4
Primary antibodies	One primary antibody fully automated. One primary antibody, or four primaries semi-automated. The Western Processor is programmed to alert the user to manually add the different primary antibodies.	
Valve delivered reagents	6 reagents	
Recover primary reagents	Yes. Second waste bottle or manual removal.	
Preprogrammed assays	5	
User-defined assays	Up to 10	
Steps per assay	Up to 15	
Cycles per step	Up to 6	
Rocking table	User selectable, 4 speed (0, slow, medium, fast)	
Volume delivery	0–500 ml	
Tubing	Medical grade Santoprene™, 1/8 inch (3 mm ID and 1 mm wall thickness) for waste: 1/16 inch (1.59 mm ID and 0.8 mm wall thickness) for feed and valve lines	
Alarm	Programmable alarm	
Timer	0–999 minutes per step/cycle	
Type of aspirate pump	DC diaphragm	
Type of delivery pump	Peristaltic	
Pump speed (delivery)	Two ml per second	
Tray (Standard & Mini-blot)	High impact polystyrene (white)	
Valves	Viton and U-polymer	
Connectors	Polypropylene	
Power Supply requirement	Globtek power supply, model#1240	
Environmental requirements	• For indoor use only • For use only up to 2000 meters • To be operated only between 5 °C and 40 °C • To be operated with a maximum relative humidity of 80% for temperatures up to 31 °C, decreasing linearly to 50% relative humidity at 40 °C • Installation category I • Pollution degree Z	
Regulatory	CE, EN61010-1	
Dimensions	26 (W) x 31 (D) x 13.7 (H) cm	

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* Two standard and eight mini blots may be processed at one time if placed in trays back to back. This may result in higher backgrounds than if processed singly.

Appendix D
Worksheet for Programming a Method for the Western Processor

METHOD NAME: _____ (Up to 5 characters)

Valve # with pre-programmed names.
Valve 1: WASH1
Valve 2: WASH2
Valve 3: CONJ1
Valve 4: SUBST (Valve 4 allows for a variable incubation time to monitor the development step)
Valve 5: CONJ2
Valve 6: REAGT
MANUL (Allows for manual addition of reagents, alarm will sound to prepare/add reagent)

Step #	**Step # Reagent Name/Valve #**	**Incubation - hr. -min**	**Variable Incubation** *Valve4, subst only*	**Volume -- ml** *Not for Manul steps*	**Rocking Speed?**	**Reagent Alarm?**	**# of cycles** *Max. 6 per step*	**Aspirate after this step?**	**Manul Aspirate Volume** *For Manul steps only*
Step 1									
Step 2									
Step 3									
Step 4									
Step 5									
Step 6									
Step 7									
Step 8									
Step 9									
Step 10									
Step 11									
Step 12									
Step 13									
Step 14									
Step 15									

Note: After running a method, it is very important to immediately wash all six (6) lines and valves using the "Purge Tubing Mode". If buffer salts and other reagents are allowed to remain in the system, the lifespan of the valves will be greatly reduced.

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