precision BIOSYSTEMS

a new standard for western blot performance

USER MANUAL

BlotCycler™ automated western blot processor.

Revision 11.0.3., 04-2017 software ver 3.25

Table of Contents

Specifications and Safety……3

Unpacking and Testing.....5

Overview 7

Programming 8

Instrument Set up and Operation…….....11

Appendix.....18

Troubleshooting 19
Fuse replacement..... 20
Packing Instruction..... 21
Accessory Products..... 23
Technical Support.....25

Specifications and Safety.

Please read this manual thoroughly before operating the BlotCycler.
We suggest that you keep this manual, as you may need to refer to it.
The BlotCycler complies with the European Community Safety requirements. Operation of the BlotCycler is subject to the conditions described in this manual. The protection provided may be impaired, if the equipment is used in a manner not specified by Precision Biosystems.

NOTE:
Notes will be used throughout the manual to inform on important points and provide useful hints.

CAUTION:
Cautions will be used to inform the reader of action that may have the potential to either harm the instrumentation or affect the quality of the data.

WARNING:
Warnings are used to provide special notice of actions that have the potential to cause harm to the operator.

For technical assistance, call, write, fax, or email.

Call: 888-490-4443 x 2
Fax: 617-812-2672
Email: customersupport@precisionbiosystems.com
Write : Customer Support
Precision Biosystems
241 Francis Avenue
Mansfield, MA 02048

Specifications:

Input voltage: 110VAC or 220-240 VAC (check unit label)
Voltage variation: +/-10%
Phase: Single phase
Power frequency: 50Hz or 60Hz (check unit label)
Rated Input current: 5.0A max
Overvoltage category: Transient overvoltage category II
Rated pollution applied: Pollution Degree 2

The device must be connected to a mains socket outlet with protective earthing connections.
- Japan
Use the cable that comes with the product.
- North America
Use the cable that comes with the product.
- Other countries
AC power cable is not attached to the product. Use a power cable that conforms to the regulations in the country where the product is to be used.

Blot Processing Polyurethane Trays and Tank.
Mini 8–25 ml per chamber, 9.5 x 7.5 cm
Midi 12–40 ml per chamber, 9.5 x 15 cm
Delta: 3-15 ml per chamber, 9.5 x 4 cm
Dimensions (h × w × d) 35 × 34 × 42 cm (with Mini tray)
Weight 12.0 kg

Safety certifications: CE directive 2006/95/EC and 2004/108/EC, standard used EN61010-1, IEC61010-1, EN 61326-1:2006, IEC 61326-1:2005

Installation location conditions

Operation site: Indoors
Maximum operating altitude: 2500 m or lower
Operating temperature 3ºC to 42ºC

WARNING:

(1) Do not operate the instrument under voltage fluctuations exceeding 10% of the recommended line voltage. Large fluctuations may cause the instrument to fail. Use a three-pronged electrical outlet with a ground.
(2) Instrument can be used in the temperature range 3°C - 42°C, avoid freezing. Do not install the equipment at a place where the temperature changed frequently.
Instrument can be used under a humidity range of 30 - 80% (RH). Relative humidity less than 80% from 3ºC to 30ºC, decreasing linearly to 50% from 31ºC to 42 ºC
(3) Do not install the equipment near a heating element.
(4) Do not install the equipment at a place where it may be exposed to corrosive gas.
(5) Do not install the instrument in a location where it may be exposed to dust, especially in locations exposed to outside air or ventilation outlets that discharge dust particles.
(6) Do not install the equipment at a place constantly or excessively exposed to oscillations or impacts.
(7) Do not install the instrument in a location where it may be exposed to direct sunlight.
(8) Avoid strong magnetic fields and sources of high-frequency waves. The instrument may not function properly near strong magnetic fields or high frequency wave sources.

The WEEE (Waste Electrical and Electronic Equipment) symbol indicates that this product should not be disposed of in unsorted municipal waste. Follow local municipal waste ordinances for proper disposal provisions to reduce the environmental impact of WEEE.

Unpacking the BlotCycler.

When you receive the BlotCycler, carefully inspect the shipping box for any damages, which may have occurred in shipping. Any damage to the container may indicate damage to its contents. Watch video how to unpack BlotCycler

Open the box and take out the enclosed flat box with tray and tank covers and tray plugs. Remove all solutions and accessories parts from the box.

Using the upper part of the green Styrofoam as a handle, pull BlotCycler out of the box and put it on a flat surface. Gently remove front and back of the protective Styrofoam; it is a good idea that you have a lab colleague help with the system removal from the box. Remove the bag. Put the instrument on a solid and leveled surface, and inspect for any damage.

Examine the unit carefully for any damage incurred during transit. If you suspect damage to the contents may have occurred, immediately file a claim with the carrier in accordance with their instruction before contacting Precision Biosystems. The warranty does not cover in-transit damage. Notify Precision Biosystems (**info@precisionbiosystems.com**) of any claim filed.

Remove the styrofoam insert between the black pumps inside tank. Put all parts back into the box and save them in case you need to send instrument back for service.

Packing List:

BlotCycler with six, five or four trays
Waste tubing (may be attached to the unit or in separate bag)
Power cord
Tray lids, 2
Tank lid, 1
Tray plugs, 4
Transformer 220-240 V /110V (optional)

Installation.

Take the power cord from the box, connect it to the back of instrument and plug into power output. Check the label on the back panel and select a correct input voltage (110 v or 220V) and frequence (50 or 60 Hz). Attached the drain tubing to back of the instrument and place the drain tubing into a waste container (not provided) or directly into the sink.

Make sure the longer tubing under the trays (see picture) is inserted into the small holes in the waste collectors on the left and right sides of the instrument.

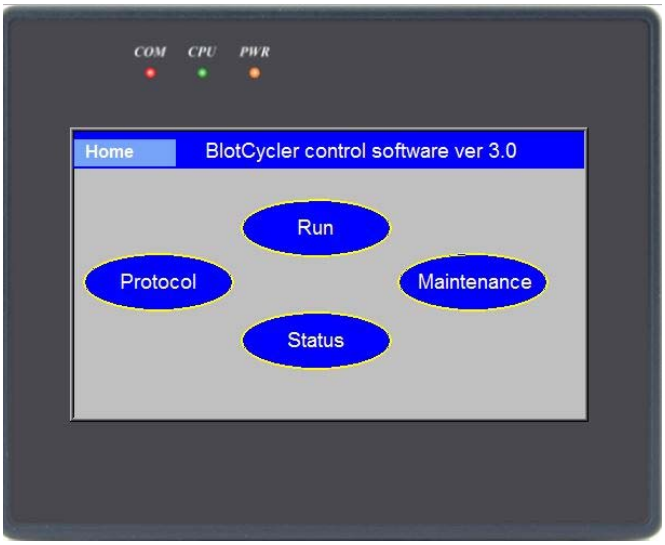
Remove protective film from the tank and tray lids.

Warning: For personal safety the BlotCycler must be properly grounded. 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. Do not under any circumstances, cut or remove the third ground prong from the power cord. Do not use a two-prong adapter plug.

Warning: Do not operate around flammable liquids or gases.

Test run

Turn on the instrument using the switch on the back of the unit. The display should light up and you should see the first screen with information about different functions:



Check that the silicone plug is inserted in the tank between the black pumps. Make sure that the waste tubing is going down and is not bent. (Remember there is no pump in BlotCycler the solution moves under gravity). **It is important for solution removal that the waste tubing is not bent allowing the solutions to drain properly, also the outlet of the tubing should not be immersed in the waste solution.**

Left side cleaning:

Fill water 2-3 cm above minimal level (about 2 L); make sure water is not leaking.

Press 

button and then press 

button, select left side. Now you started the cleaning cycle on the left side. Make sure that solution is going into the trays and out of the trays. You may stop the cleaning cycle at any time as soon you verified that everything on the left side is working.

Right Side cleaning:

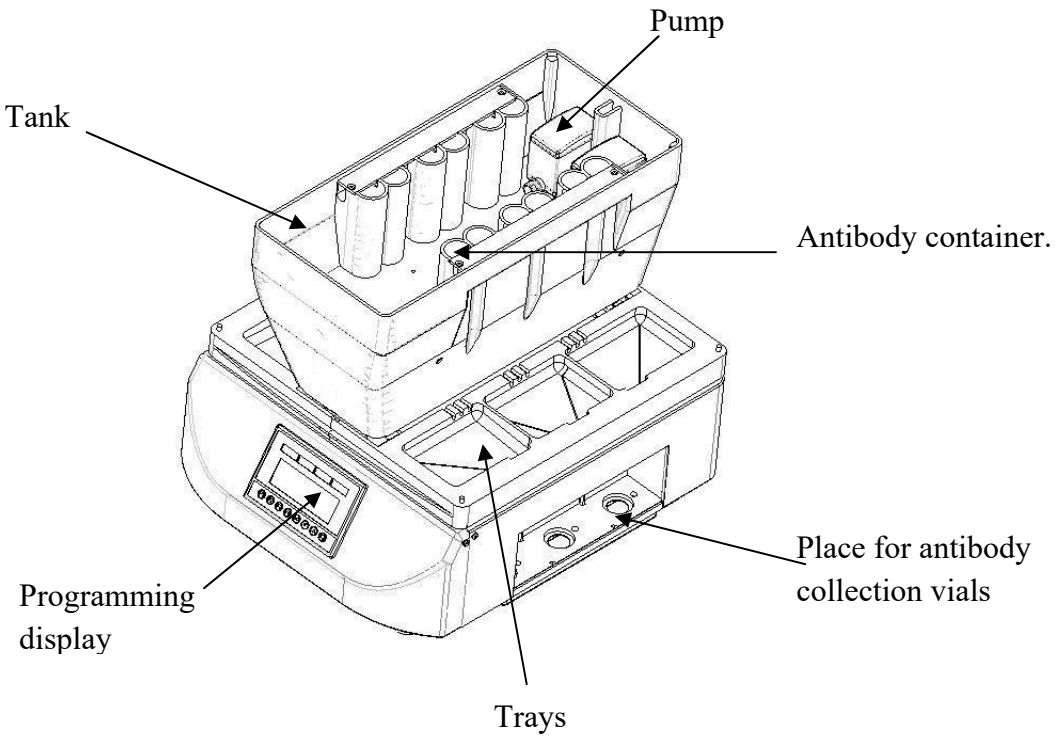
Repeat the same procedure for the right side, adding more solution if needed. Press 

button and select right side. The cleaning of the right side should start. The solution should be coming out from all three tubes in each tray.

Now the instrument is ready for use. Read the following instructions to program and start using the BlotCycler.

Overview

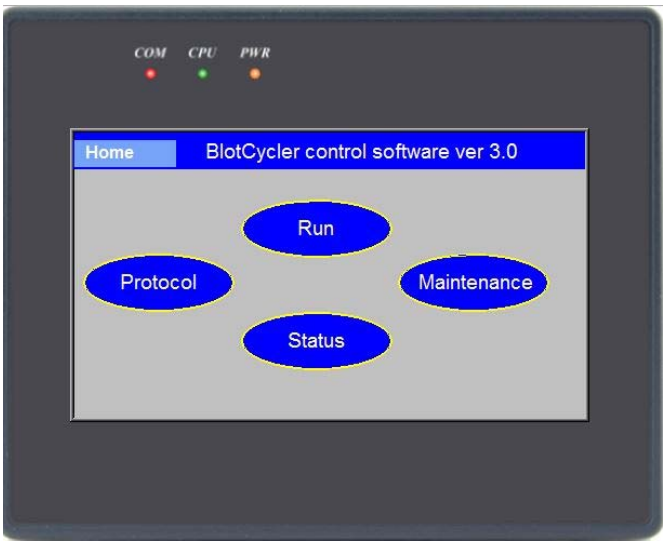
BlotCycler™ consists of programming display, four to six trays for blots (trays #1-3 on the left side and trays #4-6 on the right side), tank with container for primary (P) and secondary (S) antibodies and pumps for cleaning.



A. Programming the BlotCycler

Before starting actual western blot processing get familiar with programming interface

A1. User Interface overview



User interface contains 4 buttons:

press this button to select existing or set up a new protocol.

press this button to start a protocol

press this button to check the status of the currently running protocol and time to completion

press this button to start cleaning and perform other functions (see below)

A2. Protocol overview.

BlotCycler is set up to run standard protocol for western blot starting with blocking, washing after blocking, and then primary antibody incubation, washing after primary antibodies, secondary antibodies incubation and washing after secondary antibody incubation. Each step can be programmed independently. At the end of all steps the trays are filled with washing solution and shaking is stopped; the antibody containers are also filled with washing solution to facilitate cleaning.
***Note*:** *In order to skip blocking and primary antibody incubation set the Primary antibody incubation time to zero or press 'skip' button. Protocol will start at second washing step.*

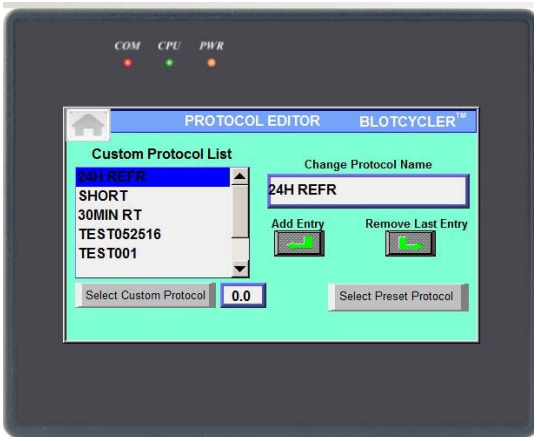
A3. Programming BlotCycler (protocol set up).

To start programming the operator needs to initialize the system by turning the system OFF and ON using the ON/OFF switch on the back of the unit. Please wait 3 sec before turning it on.

To start protocol set up press 

.

On the new screen you can select existing protocols, modify existing protocol or set up a new protocol. You can set up and save up to 20 protocols and there are 4 preset protocols that cannot be changed:



To select a preset protocol touch 

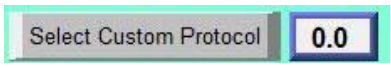
you will see a choice of four preset protocols, select one, and choose on which side(right or left) to run your protocol, a new screen to start run will appear (see below)

To add a new protocol press 

and then touch white area under "**Change Protocol Name**". Using keypad enter new protocol name.

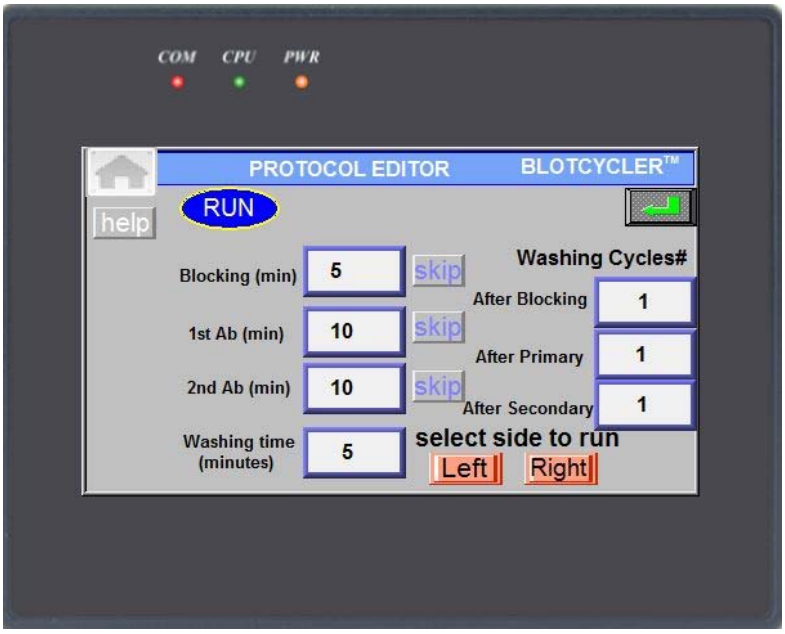
To delete last protocol press 

.

To select or modify existing protocol, highlight protocol by touching it and press 

.

On the following screen you can change any protocol parameters:



Set the incubation time for blocking, primary (PA) and secondary (SA) incubation by touching adjacent white area.

Note: The minimum time for blocking is 5 minutes, the minimum time for PA and SA incubation is 10 minutes.

Note: press 

to skip the step, zero time for blocking, PA or SA will appear.

<u>Set the number of washing cycles:</u>

By touching the white area on left side of the screen set the number of washing cycles after blocking, PA(primary Ab) incubation time and SA(secondary Ab) incubation time.

Note: you can skip washing after blocking by selecting zero, but you cannot skip washing after primary and secondary antibodies incubation.

<u>Set the duration of washing cycle:</u>

By touching the white area on left of **"washing time"** you select the duration of each washing cycle. It can be set between 3 and 20 min.

After finishing the protocol modification select side you would like to run: 

.
Please note if the button is dimmed, this side is running and cannot be selected

Press 

button on the top of the screen and use the following screen to verify protocol selection and to start protocol (see below).

B. Set up and Operation

B1. Before starting, you will need to run electrophoresis, transfer protein to membrane and prepare the following solutions:

- Blocking solution for each blot: 12-18 ml mini tray and 18-30 ml for midi tray
- Primary Antibody (PA) for each mini trays 8-15 ml , for midi trays 18-25 ml; for delta trays 3-10 ml
- Secondary Antibody (SA) for each mini trays 12-18 ml for midi trays 18-30ml, for delta trays 4-12 ml
- Washing buffer up to 3.5 L (depending on the number of blots)

Note: Use 0.1% Tween 20 in the washing, blocking and antibody buffers. This will reduce the surface tension of solutions and ensure even distribution of the antibody over the blot during incubation.

B2. Loading of the Blot Cycler

Note: If the BlotCycler has not been used for several days, run a cleaning cycle first.

- Remove tray covers, place membranes in the trays
- Add blocking buffers to each tray containing a membrane.
- Close the trays by replacing the covers

Note: tray cover has a cut that should be on the upper side to ensure trays are covered completely.

- Remove tank cover
- Add Primary Antibodies (P1 – P6). Insert collection vial for primary antibody collection and re-use

Note: Make sure that primary antibody (P) and trays are matched.
(For midi size trays you can use only 1 and 3 on the left side and 4 and 6 on right side)

- Add Secondary Antibodies (S1 – S6).

Note: Make sure that secondary antibody (S) and trays are matched.
(For midi size trays you can use only 1 and 3 on the left side and 4 and 6 on right side)

- Fill the reservoir with washer to the appropriate level, replace the top cover.
- Place a waste container, making sure it is below the instrument level and the waste tube does not touch the waste solution.

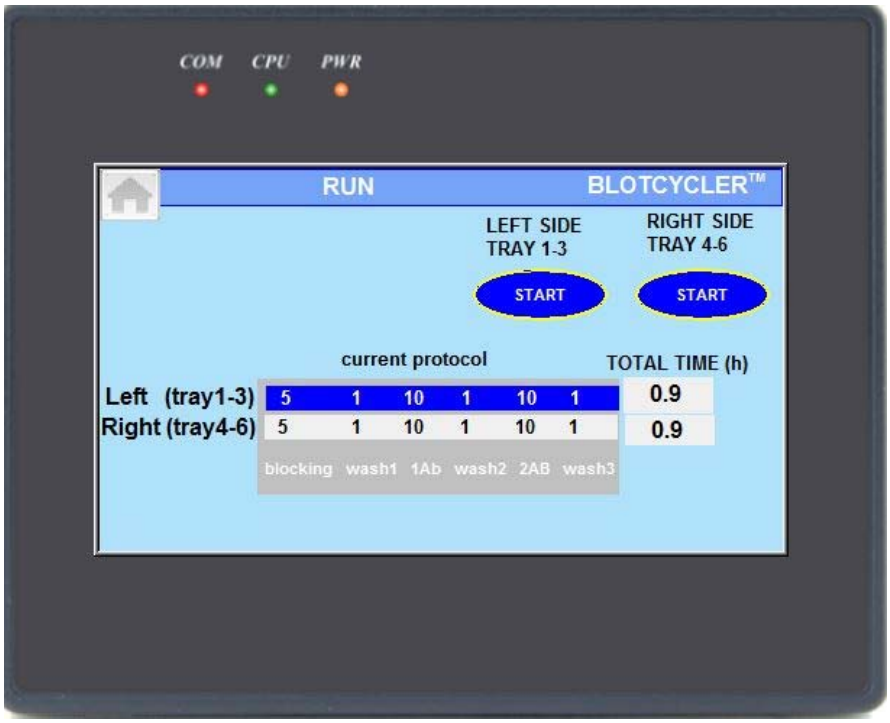
Note: If you do not use all trays you can plug washing tubing with yellow plugs provided.

B3. Start Cycling

Note: you do not need to change any settings, if you are using the same protocol as before.

- Press 

button to open a following screen:



- Press corresponding start button to start cycling for left (Tray 1-3) or right side (Tray 4-6).
  A new screen will appear that show the status of current protocol:

Red blinking dot indicates the current step, the number above it indicate the time to completion of the current step.

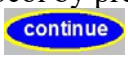
In order to return to previous screen press 

button.

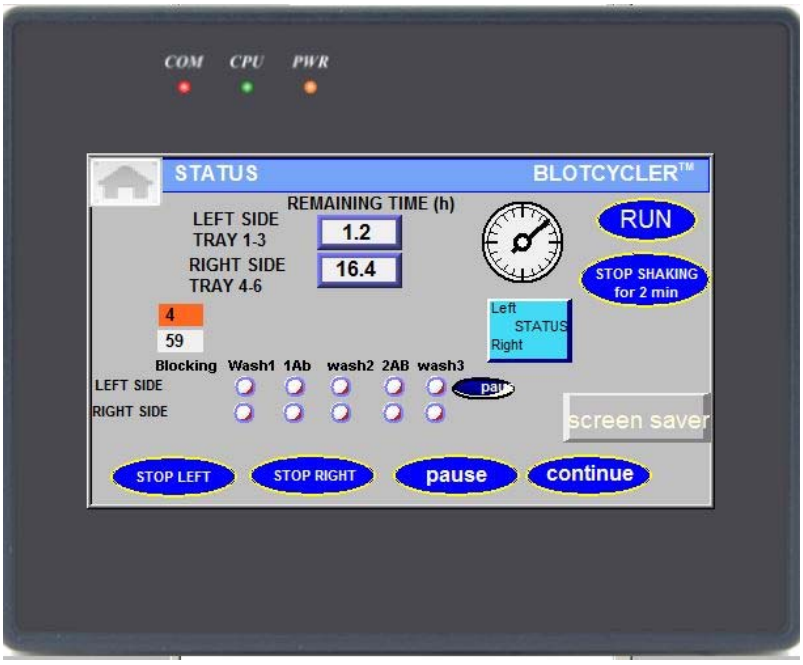
B4. Pause Cycling

- you can pause protocol by pressing 

button. Shaking will not stop

To continue protocol press 

button for left or right side.



Note: protocol will start from next step if it was close to the end of the previous step.

Use 

to stop shaking while loading blot into trays. Protocol on other side will not stop, shaking will resume in two minutes.

To completely stop protocol use 

or 

buttons

B5. Cleaning

Note: you can do a cleaning only after both sides have finished cycling.

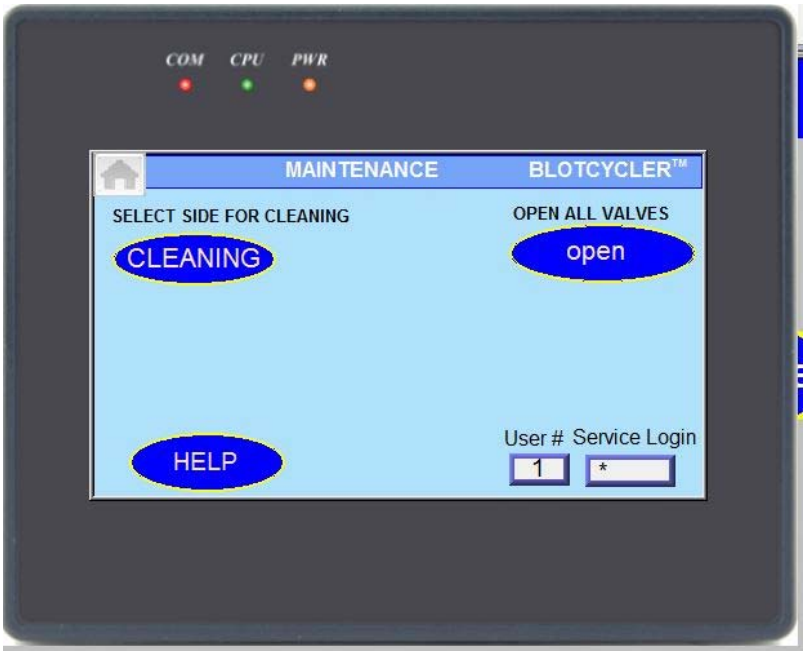
- Remove all membranes and containers for primary antibodies.
- Fill the device with cleaning solution up to Max Level (if you want a shorter cleaning cycle you can add less buffer, but at least 2 L).
-

Note: Make sure there is enough cleaning solution otherwise the pumps can be damaged.

- Go to the home screen and press 

button, a new screen will appear:

.



Press 

and select left or right side or both side on pop-up window.

Note: if you select both, left side starts first and the right side will continue after cleaning on the left side is completed.

Note: to remove the excess of cleaning solution from the tank you can repeat the cleaning cycler or just remove the plug located between the pumps.

Note: you can stop cleaning at any time but need to restart BlotCycler before starting a new protocol.

B6. Tray size setting

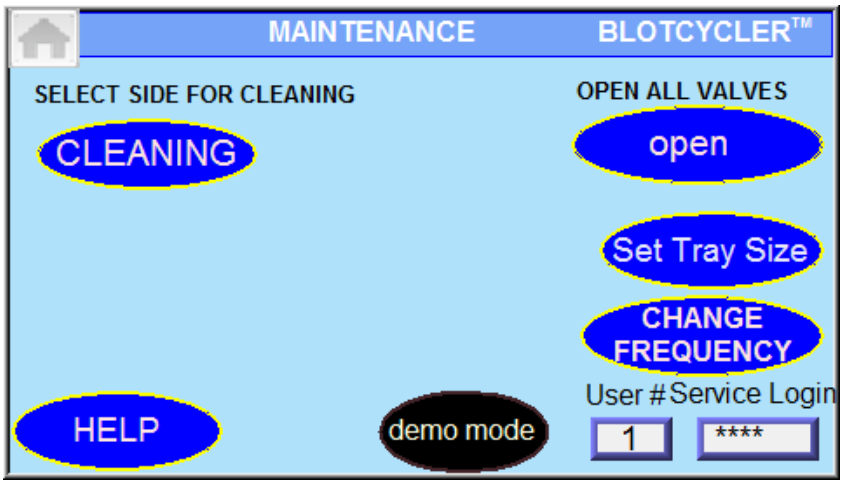
Note: Finish all protocols and remove liquid before next step. Refer to separate instruction regarding tray replacement

Go to the home screen and press 

button, a new screen will appear. Touch service login and enter password *4443*.

The new buttons appear: 

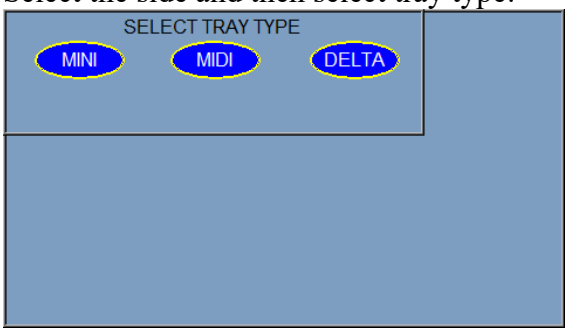
and 

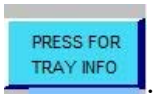


Press 

button:

Select the side and then select tray type:



Go to home scree and press STATUS button and then select 

. Make the correct tray are selected.

B7. Frequency selection (Japan only)

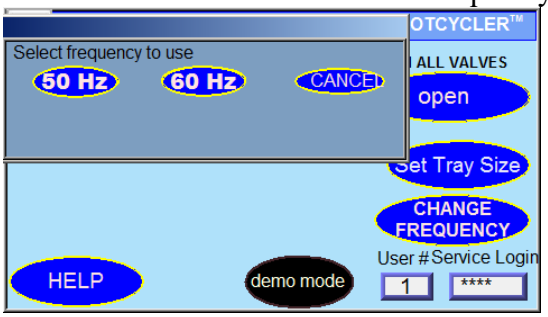
To select correct frequence for the area go to the home screen and press 

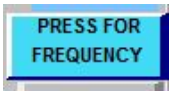
button, a new screen will appear. Touch service login and enter password *4443*.

Press 

button.

On the new screen select correct frequency for your area:



Go to home screen and press STATUS button and then select 

Verify that correct voltage frequency selected.

B8. BlotCycler Maintenance:

Run cleaning cycle every time you finished western blot processing. You can use deionized or distilled water for cleaning. At least once in a week perform intensive cleaning with cleaning solution (cat #CL500).

If the BlotCycler is not intended to be used within next 24h, run cleaning cycle with cleaning solution and then with deionized or distilled water and open all valves. It is a good idea to keep all valves open while BlotCycler is not in operation.

Go to home screen and press 

button and then 

button.

- When all valves are open, the beep sound and the message will appear. Now you can turn BlotCycler off.

Note: before opening valves check that there is no solution in the tank or the level close to minimal level.

- Now turn BlotCycler off.

Note: after opening valve you need to restart BlotCycler using switch on the back before using it again.

Warning: ***If there is a risk that a large volume of spilled liquid has penetrated the casing of the instruments and come into contact with the electrical components, immediately switch off the system, wipe out all spilled solution and do not operate until completely dry.***

Appendix

Troubleshooting

Problem	Possible Cause	Solution
No power (the digital display remains black when the power is turned on)	AC power cord is not connected.	Check AC power cord connections at both ends. Use the correct cords. Replace the fuse
	Fuse has blown.	If the problem still persists after verifying that correct power cord is used and the fuse is replaced, contact Technical support.
Buffers leak from the trays and tank immediately	Valves remain open.	Turn instrument off, wait at least 5 sec and turned instrument on. After initialization valves will be closed.
Weak or no signal from the blot	Detection step missed or detection reagents not working.	After the blot processing is complete, perform the detection step using your standard detection reagents and protocol manually. Make sure the detection reagents are functional.
	Insufficient incubation with detection reagent	Remove blot from detection reagent when signal-to-noise ratio is acceptable.
	Poor or incomplete transfer	Make sure transfer apparatus and membrane sandwiches are assembled correctly. Use appropriate transfer times. After blotting, stain membrane to measure transfer efficiency.
	Protein of interest ran off the gel	Use positive control and/or molecular weight marker to match gel separation range to size of protein being blotted. After blotting, stain membrane to measure transfer efficiency.
	Incorrect reagents added or incorrect containers are filled	Make sure that primary and secondary antibody are added to correct containers and number on antibody container in the tank and tray match each other.
	Sample too dilute	Load the larger amount of protein onto the gel or increase concentration of proteins.
	Poor retention of proteins or protein weakly bound to membrane	Use membranes with appropriate binding capacity. Dry PVDF membrane after protein transfer to ensure strong binding of the proteins.
	Inactive or overly dilute primary or secondary antibody	Determine antibody activity by performing a serial dilution using six trays or dot blot. Increase antibody concentration as necessary.
High background on the blot	Film overexposed or became wet during exposure	Decrease exposure time or allow signal to further decay. Prevent leakage of solutions by encasing membrane in transparency film and blotting excess substrate from edges before exposure.

	Short blocking time or washing intensity	Increase blocking time and the number of washes
	High concentration of primary and/or secondary antibody	Determine optimal antibody concentration by performing dilution series using all six trays. Decrease antibody concentration as necessary.
	Protein is overloaded	Reduce load or dilute concentration of sample.
	Membrane, solutions, trays, or antibody containers are contaminated	Use clean glassware and purified water to prepare solutions. Wear clean gloves at all times. Use forceps when handling membranes. Run cleaning protocol with cleaning buffer, increase the concentration of cleaning buffer two times
	Protein is overloaded	Reduce load or dilute concentration of sample.
Non-specific binding too high	Insufficient removal of SDS or weakly bound proteins from membrane after blotting	Follow proper protocol for membrane preparation before immunodetection.
	Short blocking time	Increase blocking time.
	Affinity of the primary antibody for the protein standards	Check with protein standard manufacturer for homologies with primary antibody.

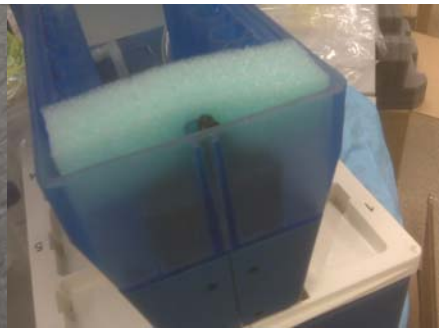
Replacing the Fuse

Follow the instructions below to replace the 250V, 5A rated fuse for the power socket.
1. Turn off BlotCycler using switch on the back of the instrument and detach the power cord from the rear of the instrument.
2. Open the fuse compartment located on the power entry block using a small flat blade screwdriver or fingernail to gently open the fuse compartment.
3. Pull the fuse holder out of the compartment and inspect the fuse. If the fuse is burned or there is a break in the fuse element, replace the 250 V, 5 A with the identical type fuse.
4. Place the fuse holder back into the compartment and snap the cover closed.

For additional fuses, contact Customer Support.

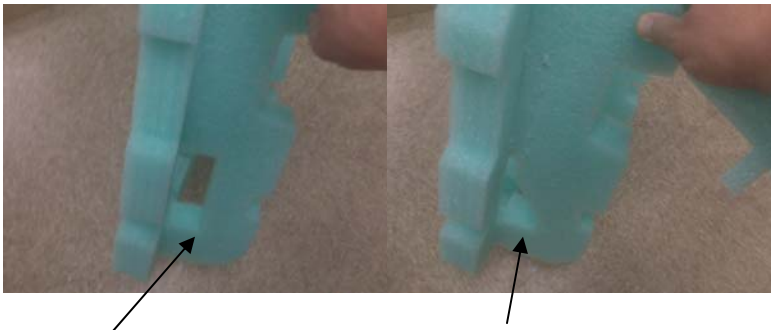
Repackaging the Instrument

Find an original box; take out an insert a flat box (originally contained tray and tank covers and dummy vials). Find a small T-bar and place between pumps into the tank.



(If you cannot find T-bar, use any appropriate material to secure pump inside tank)

There are two green Styrofoam covers: front and back (they are slightly different):



Back Front

Remove tray and cover and place them in a flat box (see below). Put on front and back sterofoam covers on the instrument. Make someone help you to make sure that instrument is not flip over.

Using upper part of green Styrofoam as handles put BlotCycler in the box:

Place insert flat box over instrument (if you cannot find flat box use any soft material to fill the gap between instrument and box top surface):

Close and tape the box.

Ship to:
Precision Biosystems
ATTN: Production Team
241 Francis Ave
Mansfield, MA 02048, U.S.A.

Accessory Products

The following products are for use with the BlotCycler and are available separately from Precision Biosystems. For more information visit www.precisionbiosystems.com or contact Customer Support.

Catalog #	Description
vi100	Antibody collection vial per 100
vi50	Antibody collection vial per 50
CL500	Cleaning solution 50x, 500 ml
CL5005	Cleaning solution 50x, 500 ml, 5 bottles
CL5010	Cleaning solution 50x, 500 ml, 10 bottles
BH500	Hybridization buffer, 500 ml
BW1000	Washing buffer 10x,1L
BW4000	Washing buffer 10x 4L
TR1003RL	Pair of Block of mini trays right & left
TR2003L	Replacement Block of midi trays left
TR2003R	Replacement Block of midi trays right
TR2003RL	Pair of Block of midi trays right&left
TRWF1015	Replacement tray for Blotcycler-Flex, midi size
TRCVC	Tray cover, clear
TNCVC	Tank cover clear
SST1001	Instrument support stand
TPL06	Tray plug, bag of 6
DRVI06	Drain (dummy) vial (pack of 6)
TRTUB12	Tray tubing replacement kit
TNTUB18	Tank tubing replacement kit
RCI002	Reagent Collector Insert
PHR02W	Electrophoresis unit, minigel size with power supply

Warranty

The BlotCycler is warranted for one (1) year against defects in materials and workmanship. If any defects should occur during this warranty period, Precision Biosystems will repair and replace the defective parts without charge.

However, the following defects are specifically excluded:

1. Defects caused by improper operation and maintenance.
2. Repair or modification done by anyone other than Precision Biosystems or their authorized agent.
3. Use with other spare parts not specified by Precision Biosystems.
4. Damage caused by deliberate or accidental misuse.
5. Damage due to use of improper solvent or sample.
6. Replacement of tray tubing.

For inquiry or request for repair service, contact Precision Biosystems.

Technical Help

For more information or technical assistance, call, write, fax, or email.

Call: 888-490-4443 x 2
Fax: 617-812-2672
Email: customersupport@precisionbiosystems.com
Write : Customer Support
Precision Biosystems
241 Francis Avenue
Mansfield, MA 02048