

GOBlot *Western Blot Processor*

Cat. # WBM01



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Patent Pending: The mechanism and design of this device is the subject of several US, PCT and other country specific patent applications.

Section I: Hazards and Precautions



Warning! Power Rating. GOBlot's power cord must be connected to a power receptacle (socket) that provides voltage and current within the specified rating for the system (110-240 Volts / 30W / 250 milli-Amp). Use of an incompatible power receptacle may produce electrical shock and fire hazards. Do not use near constant moisture or standing liquids. Do not use non-grounded plug socket for power adapter connection to wall socket.

Warning! Internal Voltage. Always turn off power switch and unplug power cord before cleaning the outer surface of the equipment. The symbol == denotes Direct Current. If external grounding is required, connect an insulated wire from the aluminum base plate (underside) to the external ground. Do not use non-grounded plug socket for power adapter connection to wall socket.

Warning! Injury to body parts. The GOBlot device contains moving cam and Reaction tray parts, avoid placing fingers, hands and other body parts into these areas.



Warning! Biohazards. Do not use biohazards with this device. Always wear safety glasses, gloves and a lab coat when working with biological reagents.

Warning! Liquids. Avoid spilling liquids on the device; fluid seepage into internal components creates the potential for short circuit and shock hazards. Wipe up all spills immediately and let the machine dry before further use. There are no hazardous gases or liquids in the device per se.

Warning! Mechanical Interruptions. In case of a mechanical interruption the device will continue running but the results might not be usual. There is a possibility of hazardous gases or liquids emanating from the device if it is intentionally or unintentionally halted during operation.

Warning! Unspecified use. Failure to use the device, parts/accessories supplied by the manufacturer or the recommended buffers and solutions in the manner described in this manual could result in hazardous conditions. Do not use the device in hazardous environments such as fume hoods. Do not use non-recommended liquids such as ethanol or acetone which could cause melting of plastics and short circuit the electrical components.

Precautions

Caution: Service. If the machine is not operating as expected, first perform the Quality Check in the troubleshooting section and if it still does not work properly, then call Cytoskeleton Inc. for technical advice. Repairs should only be performed by a Cytoskeleton technician or an authorized representative. Do not unscrew the base plate retaining screws or attempt any other maintenance or repairs, apart from the Wash Routine #5 or the Quality Check. If other maintenance or repairs are attempted, this will void the warranty.

Safety testing by independent laboratory

This device has been tested for electromagnetic interference and safety by an independent lab, METLAB Inc. USA. In this regard it has passed EMF US FCC CFR 47 Ch.1 Pt 15 B, and UL 61010-1, CSA C22.2 61010-1, EN 61326-1, IEC 61010-1, IEC 61010-2-051:2015 and IEC 61010-2-081:2015 safety directives. GOBlot is RoHS compliant, indicating the use of lead free components and connections.

Physical Specifications

Size.....	15 cm wide x 23 cm deep x 23 cm high.
Weight	1.1 Kg
Electrical rating	Power adapter, 100-240VAC 1.0 Amps. 50-60Hz (converts to 12VDC 3.34Amps). GOBlot device requires 12 VDC 1 Amp with a barrel connector with negative external and positive internal connectors, i.e.  .
Reproducibility	+/- 10% western blot band intensity.
Accuracy	+/- 10% volume dispensing.
Operating temperature range	4 to 42°C.
Operating humidity range	10 to 100% RH.
Operating altitude	0 to +2000 meters with respect to sea level.
Operating pressure range	0.8 - 1.1 Atm. (79 to 108 kPa).
Maximum pressure inside device	28 PSI.
Normal operating pressure	<5 PSI.

Section II: Warranty

The purchase of the machine comes with a full warranty (parts & labor or replacement) for up to one year after the date of purchase. After this time, please contact Technical Support for advice on options including a maintenance/repair fee or a discount on a replacement device.

Section III: Description of the GOBlot™ Western Blot Processor

The GOBlot™ Western Blot Processor is a reliable fluid delivery device for reproducibly probing blots and membranes with primary and secondary antibodies. It was developed after communication with hundreds of research scientists over the past two years. They required a cost-effective machine that would reproduce the hands-on method using a rocker platform and exchanging buffers manually.



The GOBlot™ allows the operator to load up to two membranes which will be probed with the same antibody solution. The operator loads blocking solution, primary and secondary antibodies, and chooses a program from the four pre-loaded routines and presses the start button, its as easy as that! Return after four hours to develop your blot using fluorescence, chemiluminescence or another method of detection. The machine is designed to be modular and economical so that you utilize multiple machines running at different times with different routines and different antibodies, in other words, it's very flexible for multiple users.

The equipment is composed of valves, pumps, a motorized tilting platform and a control board that coordinates timing with the application of the correct solution to the membrane or blot. It can operate at room temperature or in a cold room. All commonly used buffers are applicable, e.g. PBST and TBST. The purchase of the machine comes with a full warranty (parts & labor or replacement) for up to one year after the date of purchase.

We hope you enjoy the operation of your GOBlot and please feel free to contact us anytime with questions.

The Cytoskeleton Team.

Technical Service: tservice@cytoskeleton.com

Customer Service: cserve@cytoskeleton.com

Section IV: Components

Part #	Quantity	Description
NR55	1	Assembled GOBlot™
NR51	1	Manual
NR52	2	Reaction tray lid
NR24	2	20 ml syringe barrels
NR23	2	10 ml syringe barrels
NR29 and 26	1	LPS Model# GS40A12-P1J power supply with wall plug.
NR54	1	USB cable
NR53	1	Erasable marker

Section V: Assembly & Set-up instructions

The GOBlot™ device is shipped pre-assembled and it only needs the two syringe barrels to be complete. These act as the Primary and Secondary antibody receptacles.

Screw a 10 ml syringe into the primary antibody Luer-lock, and screw a 20 ml syringe into the secondary antibody Luer-lock. Use finger tightness, do not over-tighten.

Place GOBlot™ on a firm, dry horizontal surface, check flatness using a level placed on the lower level and adjust surface if necessary to make it level.

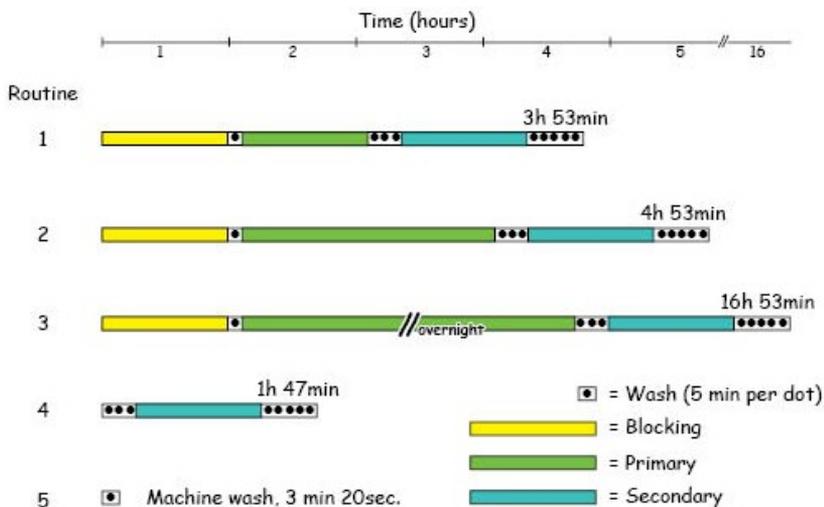
Connect the power adapter to the wall socket and to the power port at the back of the device, then turn on. Do not over extend or put the wall socket in a difficult to reach position which is a potential hazard. If disconnection is required, lift up machine, turn around and pull on the black connector. The USB port can be connected to a computer if the routines need to be recorded for internal reference purposes such as ISO9000. The USB port is not a computer control port.

To test the GOBlot before starting a western probing routine (no.s 1 to 4). Turn the Routine knob to position 5, and follow Quality Check 1 instructions in the Troubleshooting guide on p. 9. This will test the device and indicate proper operation. In the event of a power outage the GOBlot will not restart or continue from where it left off, so do not disconnect while a routine is running.



Section VI: Operating Procedure

Choose a routine from the following options



Probing Protocol

1. Prepare wash buffer, blocking solution, primary antibody solution and secondary antibody solution which are compatible with your antibody/antigen pair (see Important Notes p.8 for some examples).
2. Place the pre-wetted membrane blot and 20 ml of blocking solution into the Reaction Tray.
3. Pour 240 ml of Wash buffer (TBST or PBST) into the Reservoir Wash Tray, or 150 ml for Routine # 4.
4. Pipette 6 to 10 ml of diluted primary antibody into the front (primary antibody) syringe barrel.
5. Pipette 15 to 20 ml of diluted secondary antibody solution into the rear (secondary antibody) syringe barrel.
6. Choose the routine from numbers 1,2,3 or 4 (see above). Do not choose # 5 because this is the GOBlot's wash routine.
7. Place the lid on top of the Reaction tray to prevent dehydration and volume changes. This is especially important for small primary volumes and routine #3 which runs for 16 h.
8. Press the green start button.
9. Put a timer on corresponding to the length of time for the chosen routine. Note: In the last wash step, the buffer will not be drained from the Reaction tray, thus allowing the membrane to stay hydrated until you return.
10. Prepare the detection reagent.
11. After the routine has finished, as indicated by the cessation of Reaction tray movement, return to the device and remove the membrane from the Reaction tray. The membrane can now be treated with detection reagent. Note: Do not let the membrane dry out as this will cause background and poor signal.
12. Very carefully slide out the waste tray.
13. To re-cycle the primary antibody, pipette it out of the small chamber (see opposite) into a 15 ml Falcon tube and store at 4°C with 0.1% (w/v) sodium azide (toxic, do not use with HRP).
14. Pour the remainder of the contents in to a sink as appropriate and wash the tray with nanopure or de-ionized water.
15. Replace waste tray; be sure to perform the wash protocol after each run.

Section VII: Looking after your GOBlot

Cleaning and storage while not in use.

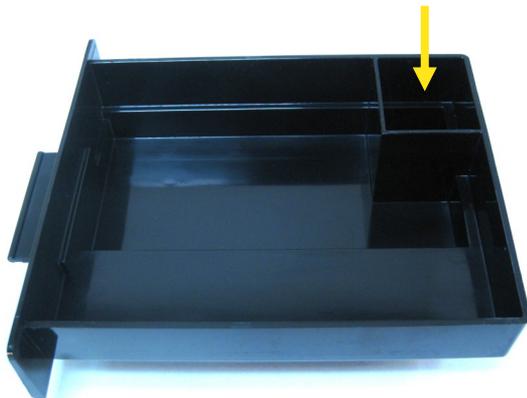
Note: It is critical to wash the device twice after each run, this will eliminate blockage due to dried buffer components clogging the tubing, pumps and valves.

Repeat this procedure twice immediately after each run:

1. Pipette all solutions out of the Wash Buffer Reservoir and the Reaction Tray.
2. Remove the waste tray and pour contents into a sink.
3. Replace waste tray and pour 50 ml of de-ionized or nanopure water into the Wash Buffer Reservoir, 10 ml into the Primary antibody syringe, 20 ml into the Secondary antibody syringe, and 10 ml into the Reaction tray.
4. Turn the Routine Knob to #5 and press the green Start button.
5. After 3 minutes return to the machine and empty the waste tray into a suitable receptacle, such as a sink for non-hazardous waste.
6. Replace the waste tray.

Note: If the GOBlot will not be used within the next week then turn off and remove the plug from the wall socket.

Waste tray indicating the primary antibody recycle chamber with yellow arrow.



Section VIII: Important technical notes

Applying liquids to the processor

It is important to pour and pipette accurately into the Wash, Primary and Secondary reservoirs because liquid overflows can result in equipment malfunction. To ensure good technique pour Wash buffer into the middle of the Wash Buffer reservoir, and for the syringe loading technique use a pipettor to pipette into the syringe at a depth that is below the rim.

Blocking solution

The most generally applicable blocking solution is TBST with 5% (w/v) dried milk powder. However other blocking reagents are possible such as 1% BSA or 1% non-specific IgG. TBST is Tris buffered saline with Tween 20 which contains 10 mM Tris-HCl pH 7.0, 150 mM NaCl and 0.05% (v/v) Tween 20.

Primary antibody solution

The best volume of primary antibody is 10 ml, at this volume all routines run efficiently and will not be subject to the effects of evaporation. If smaller volumes are required it is possible to use down to 5 ml for Routines 1, 2 and 4, or 7 ml for Routine 3. The effects of too little primary antibody volume are reduced signals and increased background at the distal end of the membrane away from the axis of tilt.

Dilute primary antibody in TBST or if this concentration produces high background then use TBST with 1% milk.

Secondary antibody solution

The secondary antibody is raised to the primary IgG species and usually is conjugated to horse-radish peroxidase (HRP) or alkaline phosphatase (AP).

Dilute secondary antibody 1:20,000 in TBST or if this concentration produces high background then use TBST with 1% milk.

Cold room operation

The GOBlot™ can be operated in a cold room at 4°C, but it is not recommended to move the machine to room temperature in the middle of a routine because if the power is disconnected the program will need to be re-started from the beginning, hence routines should be run at one temperature.

One step incubations

Routine #4 is used to incubate with just one reagent. An example is where the blocking and primary steps have been completed overnight in a cold room and the secondary antibody is left to incubate in the morning at room temperature in the GOBlot™.

Modifying the standard routines

The standard protocols can only be modified by a trained software engineer. Cytoskeleton provides a fee for service to modify the routines to your requirements, please contact tservice@cytoskeleton.com for a rapid quote.

Repairing the GOBlot™

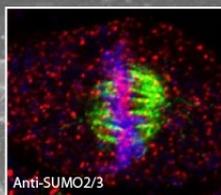
Do not attempt to repair the GOBlot. If needed, the device should be returned to Cytoskeleton Inc. for new parts. If the warranty is still valid then the repairs will be free of charge, otherwise a Purchase Order or credit card is required for the repair and parts fee.

Section IX: Troubleshooting

- Quality Check 1:** Turn the Routine knob to routine #5 (Machine Wash), pipette 10 ml of Wash buffer into the Primary syringe barrel, 20 ml into the Secondary syringe barrel, 20 ml into the Reaction tray and 50 ml into the Wash Reservoir. Press Start button and wait 3 min for the routine to finish. Now note whether 1. the Wash Reservoir has mainly emptied, 2. the Primary and Secondary Syringe barrels have emptied, 3. the Action tray has emptied, and 4. the Waste tray contains approx. 10 ml of liquid in the recycle compartment and the rest of the liquid in the main compartment. If necessary contact tservice@cytoskeleton.com with the results.
- Quality Check 2:** If the power light does not illuminate, then check the power adapter to see if its green power light is on, then check the connections to the power socket on the device. If there is still no Power light illuminated then there may be a problem with the device, contact customer service for a repair. If the power adapter light is not illuminated then test the wall socket for power with another device. If there is power in the wall socket then possibly the power adapter has malfunctioned then contact customer service for a repair.
- Quality Check 3:** If a routine does not start (i.e. the Reaction tray should tilt up and down immediately), then turn off the machine at the rear and restart it. If there is still no routine running then contact customer service for a repair.
- High background on one end or corner of the blot. This is caused by too little antibody solution covering the membrane. This can be caused by a smaller than recommended amount of primary being used or the machine not being level on a surface. Check the volume applied to the antibody reservoirs and use a level to check the flatness of the lower level (see p.5 Assembly and Set-up).

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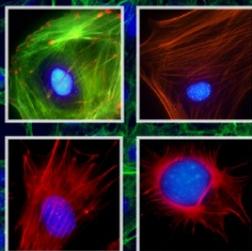
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