
Trans-Blot® Turbo™ Blotting System

Instruction Manual

Catalog Number
170-4155



BIO-RAD

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Safety Warning Labels



Warning: Do not open or attempt to repair the Trans-Blot® Turbo™ instrument or cassettes. Doing so will void your warranties and can put you at risk for electric shock. Return the Trans-Blot Turbo instrument or cassettes to the factory (US customers) or to an authorized distributor (all other customers) if repairs are needed.



Under normal operating conditions, Trans-Blot Turbo cassettes may become warm during transfer.

Safety Compliance

This instrument has been tested and found to be in compliance with all applicable requirements of the following safety and electromagnetic standards:

- EN61010-1 Electrical Equipment for Measurement, Control, and Laboratory Use
- UL STD No. 61010A-1 Electrical Equipment for Measurement, Control, and Laboratory Use, Part 1: General Requirements
- CAN/CSA C22.2 No. 61010-1-04 Safety Requirements for Measurement, Control, and Laboratory Use, Part 1: General Requirements (includes Amendment 1)
- IEC 61010-1 Safety Requirements for Measurement, Control, and Laboratory Use, Part 1 General Requirements

Electromagnetic Compatibility (EMC)

EN61326 Class A Electrical Equipment for Measurement, Control, and Laboratory Use, General Requirements

FCC Warning and Notes

Warning: Changes or modification to this unit not expressly approved by the party responsible for compliance could void the user's authority to operate the equipment.

This Bio-Rad instrument is designed and certified to meet EN61010* and the EMC requirements of EN61326 (for class A) safety standards. Certified products are safe to use when operated in accordance with the instruction manual. This instrument should not be modified or altered in any way.

Alteration of this instrument will:

- Void the manufacturer's warranty
- Void the EN61010 safety certification
- Create a potential safety hazard

* EN 61010 is an internationally accepted electrical safety standard for laboratory instruments.

Bio-Rad Laboratories 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 Laboratories or an authorized agent.

We strongly recommend that you follow the safety specifications listed in this section and throughout this manual. Use only the supplied power cord with the instrument, making sure to choose the plug adaptor that corresponds to the electrical outlets in your region.

Specifications

Trans-Blot® Turbo™ Instrument

Dimensions: (L x W x H) 26.0 x 21.1 x 20.4 cm

Weight: 8 lb with cassettes, 4.5 lb without cassettes

Input power: 100–240 VAC, 276 VA, 50–60 Hz, 175 W max

Fuses: Two 6.3 A, 250 V, fuses located above the power connection

On/off switch: Yes, mains connected

USB port: Yes, input only, for firmware updates

Cooling fan: Yes

Output power: 0–26 VAC (1 V increments), 0–2.6 A DC (0.1 A increments) for each cassette

Operating conditions: 15–31°C ambient temperature, 0–95% relative humidity (noncondensing)

User Interface

18 button keypad, 128 x 64 pixel monochrome display

Programmable methods: Up to 25 user defined

Preprogrammed methods: Standard SD, 1.5 mm gels, High MW, Low MW, Mixed MW, 1 Mini-PROTEAN® TGX™ gel

Audible alarm: Yes

User notifications:

- Power fail during run
- No load detection
- No cassette detection
- End of run
- Watt/hr limit

Trans-Blot Turbo Cassettes

Dimensions: (L x W x H) 20.2 x 16.0 x 4.5 cm

Anode: Platinized titanium electrode plate

Cathode: Stainless steel

Weight: 1.5 lb

Gel Compatibility

Suitable for transfer of two mini format gels or one midi format gel per cassette. Other gel sizes can be trimmed to fit the consumable transfer packs.

Trans-Blot Transfer Packs

Tray dimensions (L x W): 18.0 x 14.5 cm

Stack dimensions (L x W): Mini format (7.0 x 8.5 cm), midi format (13.5 x 8.5 cm), + tab

Pad materials and buffer: proprietary

Membrane: 0.2 μm nitrocellulose or 0.2 μm PVDF

Note: Tray base is polyethylene terephthalate glycol (PETG) and can be recycled according to local regulations for class 1 plastics.

Trans-Blot Turbo RTA Transfer Kits

Membrane dimensions (L x W): Mini format (7.0 x 8.5 cm), midi format (13.5 x 8.5 cm)

Membrane: 0.2 μm nitrocellulose, 0.2 μm PVDF, or 0.45 μm low fluorescent PVDF

Pad materials and buffer: proprietary

Gel tray: two reusable trays to wet and equilibrate membrane and pads

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Section 1: Introduction



Fig. 1 Trans-Blot® Turbo™ blotting system.

Semi-dry western blotting is a common technique in many research and diagnostic laboratories. Conventional semi-dry blotting protocols are often cumbersome, requiring a great deal of time-consuming reagent preparation and setup, followed by an electrophoretic transfer that could take up to an hour or more. Bio-Rad's Trans-Blot Turbo system accelerates the semi-dry blotting process without sacrificing performance. With the Trans-Blot Turbo system, transfer time is reduced to as little as 3 minutes, and the prepackaged transfer packs provide excellent transfer efficiency and reproducibility while eliminating the time and mess associated with traditional transfer methods.

The Trans-Blot Turbo system is comprised of the main instrument with two blotting cassettes and single-use prepackaged transfer packs that contain buffer-saturated membranes and ion reservoir stacks.

Each blotting cassette has a built-in pair of anode and cathode electrode plates and can accommodate one or two mini format (7.0 x 8.5 cm) gels or a single midi format (13.5 x 8.5 cm) gel. Closed cassettes lock to hold a gel in contact with a transfer membrane placed between two ion reservoir stacks. The loaded cassettes are simply inserted into the instrument to access the power supply for protein transfer. A preprogrammed user-defined transfer protocol is easily selected from the firmware menus using the Navigation and Selection buttons and the alphanumeric keypad. During the run, transfer conditions and run progress are displayed on the LCD screen.

The buffer saturated ion reservoir stacks and membrane are available as ready-to-use prepackaged, disposable, single-use Trans-Blot Turbo Transfer Packs in two sizes, for transfer of single mini gels (7.0 x 8.5 cm) and a larger size (13.5 x 8.5 cm) for transfer of midi gels or simultaneous transfer of two mini gels (7.0 x 8.5 cm). Both the mini and midi transfer packs are available with either nitrocellulose or PVDF membranes. These prepackaged transfer packs allow for efficient transfer in as little as 3 minutes. Gels can be transferred immediately after electrophoresis without equilibration. The reagents and consumables available in the transfer packs are also available in ready-to-assemble format, as part of the Trans-Blot Turbo RTA transfer kits. The system will also accommodate traditional semi-dry western blotting consumables such as filter paper and Towbin buffer with efficient transfer in 30–60 min.

Selection of transfer protocols is simple and the system is designed to handle multiple sequential runs with no intervals of delay except for the reloading of the cassettes.

Unpacking and Setup Instructions

The Trans-Blot Turbo starter kit, catalog #170-4155, includes the main unit, two cassettes (bases and lids), blot roller, standard power cord, quick start guide, instruction manual, and a mixed assortment of nitrocellulose and PVDF membranes in mini and midi sizes.

Place the system on a level surface with at least 6 cm of clearance in the back for proper ventilation. Plug the power cord into the back of the instrument, and then connect it to a standard grounded outlet. Remove the foam ring separating the instrument and cassettes. Power on the instrument using the switch on the right side of the unit.

Note: Do not place heavy items on the top of the Trans-Blot Turbo system.

Chemical Compatibility

The Trans-Blot Turbo system and cassette components are not compatible with strong acids or bases, chlorinated hydrocarbons (for example, chloroform), aromatic hydrocarbons (for example, toluene, benzene), or acetone. The cassettes and instrument casing can be cleaned with water and a mild detergent, but do not use abrasives or organic solvents. The stainless steel cathode electrode, which is housed in the cassette lid, can be cleaned with 7% acetic acid and wiped down with water.

Safety/Cautions/Warning

The following guidelines should be observed and followed when using the Trans-Blot Turbo instrument.

The Trans-Blot Turbo instrument has been tested for operation at 15–31°C ambient temperature and 0–95% relative humidity (noncondensing). Operating the unit outside these conditions is not recommended.

- To ensure adequate cooling, be sure that there is at least 6 cm of clearance behind the unit and that the fan vent at the rear of the unit is not blocked
- Always connect the unit to a grounded AC outlet using the power cord provided
- Use caution when removing a cassette from the unit after a transfer run. The cassette may be warm to the touch
- Do not operate in extreme humidity (>95%) or where condensation can affect the internal electrical circuits of the unit
- Operation of the Trans-Blot Turbo system at temperatures <15°C is not recommended. However, the unit can function in a cold environment and can be operated immediately. When returning the unit to normal conditions, allow it to equilibrate to room temperature before use
- For your safety and for the protection of your Trans-Blot Turbo instrument, Bio-Rad suggests you routinely clean the instrument in accordance with the enclosed instructions and that you routinely check the contact pins located at the back of the instrument cavity, as outlined in Appendix A.
- Bio-Rad recommends that you rinse and dry the cassette base following each use of the instrument by rinsing each cassette, base, and lid in deionized water to remove residual salts and to prevent salt buildup. Air-dry or use a paper towel to dry the cassette.
- Do not add excess or additional buffer to the transfer cassette at any point prior to running the instrument.

Important: 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 emissions from this product may interfere with some sensitive appliances when placed nearby or on the same circuit as those appliances. The user should be aware of this potential and take appropriate measures to avoid interference. This product is designed and certified to meet EN 61010* safety standards. Certified products are safe to use when operated in accordance with the instruction manual. This safety certification does not extend to accessories that are not EN 61010 certified, even when used with this unit.

This instrument should not be modified or altered in any way. Alteration of this instrument will void the manufacturer's warranty, void the EN 61010 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.

* EN 61010 is an internationally accepted electrical safety standard for laboratory instruments.

Warranty

The Trans-Blot Turbo blotting system and associated accessories are covered by a standard Bio-Rad Laboratories warranty. Contact your local Bio-Rad Laboratories representative for the details of the warranty.

Section 2: Equipment and Reagents Overview

The Trans-Blot® Turbo™ instrument and reagents are designed to provide fast, efficient, and reproducible western blots of protein gels in as little as 3 minutes. Each convenient transfer pack contains the necessary materials to efficiently transfer a single mini gel (7.0 x 8.5 cm) or one midi or two mini gels (13.5 x 8.5 cm). The transfer packs contain two buffer-soaked ion reservoir stacks along with either a prewetted nitrocellulose or PVDF membrane. The membranes do not require any pretreatment before use.

The reagents and consumables available in the transfer packs are also available in a ready-to-assemble format as part of the Trans-Blot Turbo RTA transfer kits. These kits provide the same optimized buffers and consumables as the transfer packs, but require wetting of the membranes and filters before use.

Note: Traditional semi-dry western blotting consumables can also be used with the system. See Section 3.7, page 15 for details.



Fig. 2. Trans-Blot Turbo instrument.



Fig. 3. Cassette base and lid. Reversible lid is labeled with lock, unlock, and safety hazard symbols.

For transfer, a gel is sandwiched between two ion reservoir stacks in the cassette. Each cassette can hold 1 or 2 mini format gels (7.0 x 8.5 cm) or one midi format gel (13.5 x 8.5 cm). One or both cassettes can be used for a blotting run. If both cassettes are run, they must use the same protocol and have the appropriate combinations of gels, as shown in Table 1.

The cassettes can be run individually or simultaneously with independent start times using the same protocol. This allows the user to perform multiple sequential runs without interruption, except for reloading a cassette. At the end of each cassette run, the ion reservoir stacks are discarded. Membranes and gel (if needed) can be used immediately for downstream applications or stored for later use.

The cassette bays are labeled A and B for convenience, and the cassettes are freely interchangeable between the bays. There is no preference as to which bay contains which cassette when running the combination of 1 midi and 2 mini gels. Also, one bay (either A or B) can remain empty during a run.

Table 1. Acceptable gel transfer combinations when both bays are in use.

	Acceptable Gel Combinations		Unacceptable Gel Combinations	
	1	2	1	2
Upper bay A	1 mini format gel	2 mini or 1 midi format gels	1 mini format gel	2 mini or 1 midi format gels
Lower bay B	1 mini format gel	2 mini or 1 midi format gels	2 mini or 1 midi format gels	1 mini format gel

2.1 User Interface

The user interface consists of an LCD menu screen on the front of the system. Three buttons below the screen are used to navigate among menu screens. Three buttons to the right of the screen are for user selections. A standard alphanumeric keypad is used for input of text and numbers.

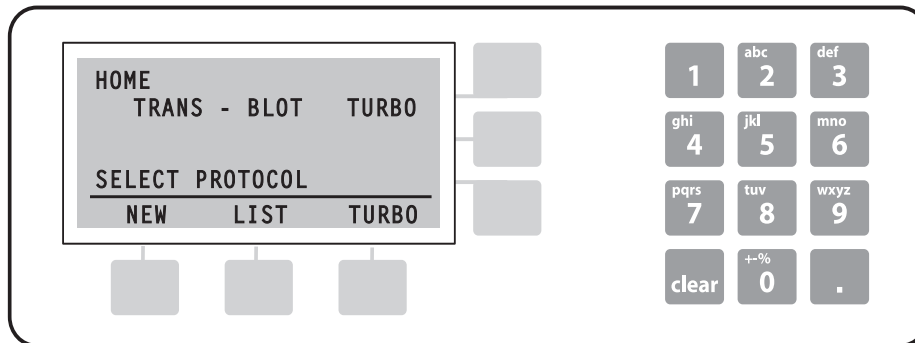


Fig. 4. Trans-Blot Turbo system user interface.

From the Home menu, three modes are available:

- **NEW mode.** Create, run, and save a new protocol for your protein of interest
- **LIST mode.** Select from either a list of Bio-Rad preprogrammed protocols optimized for a variety of protein and gel types or a list of user-defined protocols
- **TURBO mode.** Quick access menu for transfers of mixed MW proteins (MW 5–150 kD). Designed for efficient transfer of a wide variety of proteins over a broad range of molecular weights

Section 3: Trans-Blot® Turbo™ System Transfer Setup

The Turbo protocol, when combined with Trans-Blot transfer packs, provides highly efficient transfer for a wide variety of proteins. However, run conditions may need to be adjusted for a particular protein of interest. Protocols optimized for different protein and gel types are available in the Bio-Rad preprogrammed protocols in LIST mode.

The system may also be used with Trans-Blot Turbo RTA transfer kits, which offer the same transfer speed and efficiency as the transfer packs, or superior performance to traditional semi-dry blotting consumables such as extra-thick filter paper and Towbin transfer buffer (speed and efficiency will be similar to standard semi-dry blotting techniques). Please refer to the Bio-Rad Protein Blotting Guide, bulletin 2895, for more information on the methods of electrophoretic transfer. The Protein Blotting Guide can be downloaded from our website, www.bio-rad.com, as a PDF file; or call Technical Support at 1-800-424-6723 to request bulletin 2895.

3.1 Transfer Using Transfer Packs

Recommendations:

- Wear gloves at all times during the blotting process to prevent contamination of the gels or membranes
- Transfer gels immediately after electrophoresis without an equilibration step
- The transfer membranes and stacks of the transfer packs are prewetted and do not require any further pretreatment. Open the transfer packs immediately before use to avoid drying the membrane
- Use only the appropriate combinations of gels, as shown in Table 1
- If you need to move the membrane, carefully use flat tweezers or forceps

Select the appropriate transfer pack for your application according to Table 2. These packs are stable for several months at room temperature. However, to extend their shelf life, store them at 4°C. The transfer membranes and stacks of the transfer packs are prewetted and do not require any further pretreatment (for example, wetting of the PVDF membrane).

Table 2. Transfer pack options.

Gel Size (cm)	Catalog Numbers with 0.2 µm Nitrocellulose Membrane	Catalog Number with 0.2 µm PVDF Membrane
For one mini format gel, 7.0 x 8.5	170-4158	170-4156
For one midi format gel or two mini format gels, 13.5 x 8.5	170-4159	170-4157

Mini Transfer Pack (Single Mini Gel) Setup

The packaging for the mini transfer pack has the two ion reservoir stacks in the left and right wells of the tray. The right well contains the stack for the anode (below the gel) and is layered with the transfer membrane (nitrocellulose or PVDF). The left well contains the stack for the cathode (above the gel) (Figure 5). Use the finger wells of the tray to access the stacks and easily lift them from the tray. The text above each finger well signifies the stack location in the cassette. The stack and membrane in the right finger well, labeled Bottom (+) is placed on the anode in the cassette base. The stack in the left finger well, labeled Top (-) is placed on top of the transfer gel, closest to the cathode.

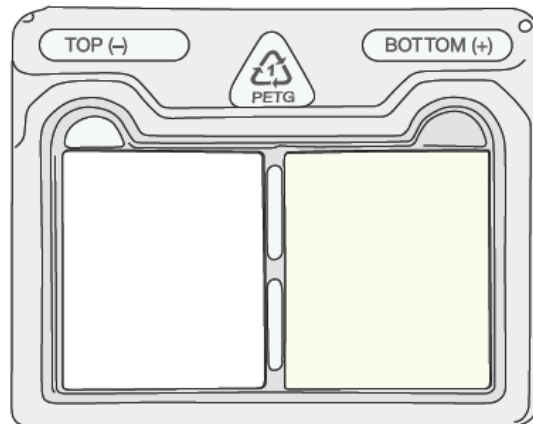


Fig. 5. The anode stack with the membrane is on the right, while the cathode stack is on the left.

Midi Transfer Pack (Single Midi Gel or Two Mini Gels) Setup

The packaging for the midi transfer pack has a tray containing the two ion reservoir stacks placed on top of each other in the tray. The ion reservoir stack on the top has a tab on the right side and is for the anode (below the gel). This stack is layered with the transfer membrane. The ion reservoir stack for the cathode (above the gel) is located below the membrane and anode stack and has a tab on the left side (Figure 6). Use the finger wells and tabs to access the appropriate stack and lift it from the tray. As with the mini trays, the text above the finger wells signifies the stack location in the cassette. The top stack and membrane, with the tab on the right, is labeled Bottom (+) and is placed on the anode in the cassette base. The stack below the anode stack, with the tab on the left, is labeled Top (-) and is placed on top of the transfer gel, closest to the cathode. For transferring two mini gels simultaneously, arrange the gels so that the foot of the gels (low-molecular weight side) face each other on the membrane. The gels will have to be placed longitudinally on the stack (Figure 11).

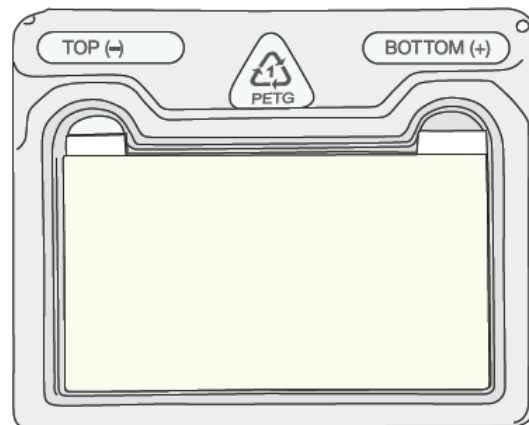


Fig. 6. The anode stack with the membrane is on the top while the cathode stack lies beneath the anode stack and membrane.

The transfer packs are vacuum sealed. Check that the membrane does not lift away with the foil packaging lid. If necessary, hold the edge of the membrane and peel while opening the pack.

Note: The base of the tray is PETG plastic and can be recycled according to local regulations for class 1 plastics.

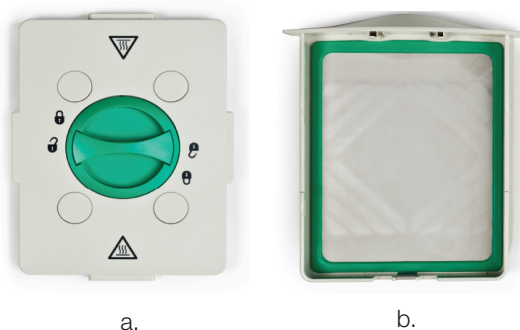


Fig. 7. Open cassette. **a.** Lid with cathode (-) on the underside. **b.** Base with anode (+).

3.2 Placing a Transfer Pack into the Cassette

See Figures 9 and 10 for step-by-step instructions to place a transfer pack into a cassette.

- The membranes and stacks are designed to be lifted from the tray and placed in the cassette. Do not invert the stacks when removing them from the tray
- The assembled transfer packs should be reasonably centered and flat within the base of the cassette (anode)
- We do not recommend moving the stacks once they are placed, as this may introduce air between the layers. If the stack must be moved after placement, use a blot roller to expel any trapped air bubbles.
- Each stack uses multiple layers of material for maximum transfer efficiency. Removing stack layers is not advised
- Refrain from adding any extra transfer buffer to the cassette, saturated transfer stacks provide ample transfer buffer.

Figure 8 shows the proper final assembly of the blotting sandwich in the cassette and is applicable to all transfer packs.

- If the gel and ion reservoir stacks are layered out of order during assembly of the blotting sandwich, carefully disassemble the components so as not to damage the membrane. Reassemble the stack and use the blot roller to remove air bubbles between the components

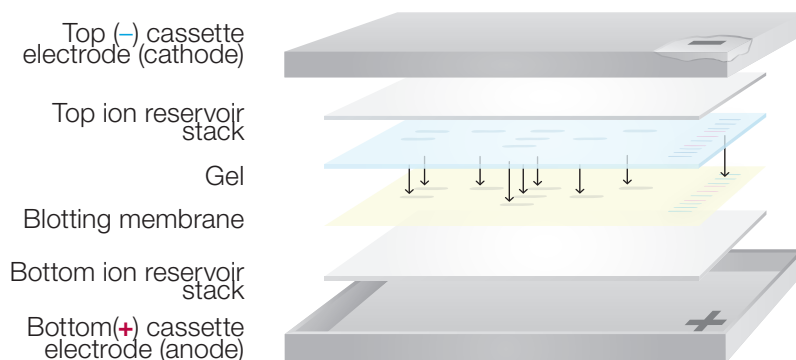
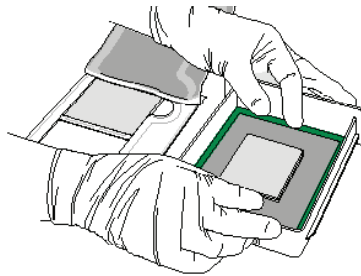


Fig. 8. Proper layering of the assembled transfer pack.

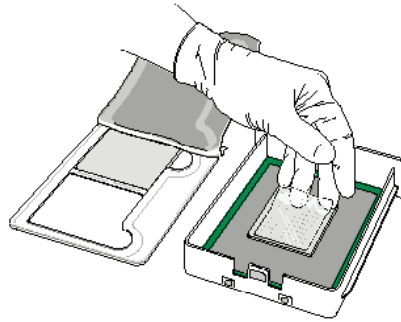
**Mini Transfer Pack
(for one mini format gel)**

a. Lay the ion reservoir stack with the membrane (anode stack) in the center of the cassette base. Ensure that the stack is not overlapping the green rubber molding in the base.



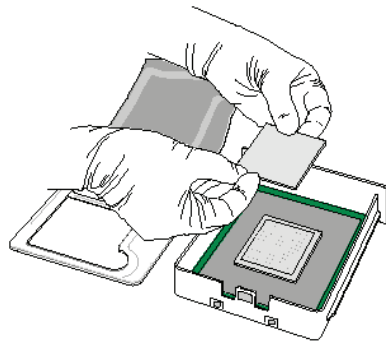
a.

b. Carefully align the gel on the membrane. If necessary, gently use the blot roller to remove air bubbles between the gel and membrane. If transferring two mini gels, place them on the membrane so that the feet of the gels are facing toward each other.



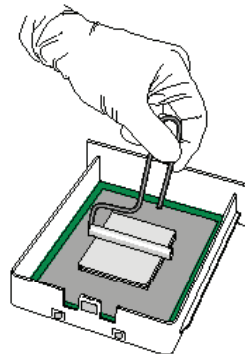
b.

c. Gently place the second ion reservoir stack (cathode stack) on the gel.



c.

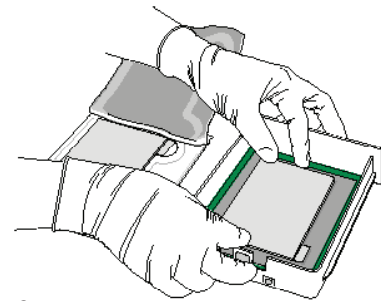
d. Use the blot roller to remove any air bubbles in the assembled transfer pack and provide consistent contact between the layers.



d.

Fig. 9. Assembling the mini format transfer pack.

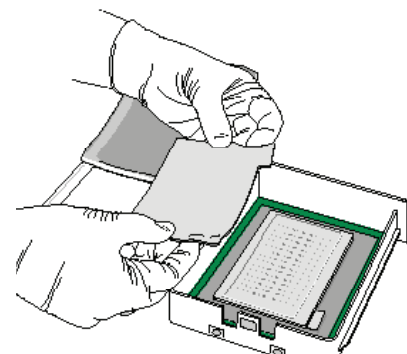
**Midi Transfer Pack
(for one midi format gel or
two mini format gels)**



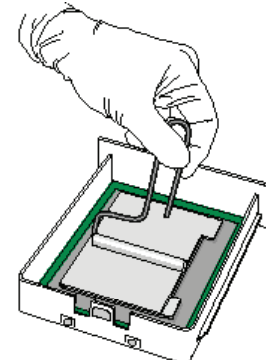
a.



b.



c.



d.

Fig. 10. Assembling the midi format transfer pack.

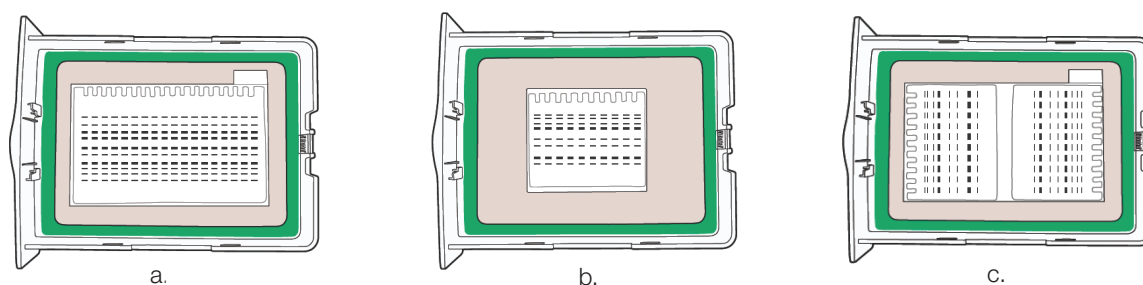


Fig. 11. Suggested placement of assembled transfer stacks in a cassette. **a.** Midi stack and gel placement. **b.** Mini stack and gel placement. **c.** Two mini gels on a midi stack.

Set the transfer stack close to the center of the cassette. A midi stack only fits along the long axis of the cassette. It is not necessary to move an assembled transfer pack if it is only slightly off center in the cassette.

Once the stacks are positioned in the cassette base, place the cassette lid on the base. The lid is reversible, but ensure that the electrical contacts fit closely into the slots in the base. Press the lid down firmly and turn the dial clockwise to engage the lid pins into the locking slots.

Slide the cassette (with the dial facing up) into one of the Trans-Blot Turbo instrument bays until it makes contact with the magnetic interlock in the back of the instrument tub and you hear a click. The cassette can be inserted into the bays with or without power to the system.

The bays are labeled A (top) and B (bottom), and cassettes can be inserted interchangeably into the bays. There is no requirement for both cassettes to be inserted; one bay (either A or B) can be left empty when a protocol is run.

The instrument is now ready to begin a transfer protocol. Refer to the following sections for details on transfer using preprogrammed and user-defined protocols.

Note: A transfer for one cassette can be started while you are assembling a second cassette. The second transfer can be started independently as long as the same protocol is being used for both.

3.3 Transfer Using RTA Transfer Kits

Trans-Blot Turbo RTA transfer kits provide the same reagents and consumables available in the transfer packs, but in a ready-to-assemble format. The protocol for using them is similar to that used for the transfer packs, except membranes and transfer stacks must be soaked in Trans-Blot Turbo transfer buffer before use. Do not equilibrate the gel before transfer.

1. Prepare the Trans-Blot Turbo Transfer Buffer according to instructions on the bottle.
2. Wet and equilibrate the membrane and transfer stacks:
 - Nitrocellulose membrane — immerse in 30 ml of 1x transfer buffer and equilibrate for 2–3 min
 - PVDF and LF PVDF membranes — immerse in 100% methanol or ethanol (use reagent grade ~85% or molecular biology grade ~95%–98% purity) until the membrane is translucent. Transfer to a soaking tray containing 30 ml of 1x transfer buffer, ensure the membrane is submerged, and equilibrate 2–3 min
 - Transfer stacks —
 - Midi stacks — immerse two stacks separated by blue sheets in two soaking each containing 50 to 70 ml of transfer buffer for 2–3 min
 - Mini stacks — immerse two stacks separated by blue sheets side-by-side in a soaking tray containing 50 to 70 ml of transfer buffer for 2–3 min
2. Follow the procedure described in Figures 8-10 above for assembling the transfer sandwiches in transfer cassettes, followed by an additional step: once assembled, remove excess transfer buffer by inverting the cassette base with the assembled stack carefully held in place into a waste container. Once the excess buffer is removed, place the cassette lid on the base, and proceed with the transfer step (3.4).

3.4 Transfer Using the Turbo Protocol

The Turbo button immediately accesses the MIXED MW program. It is designed for efficient transfer of a wide variety of proteins over a broad range of molecular weights. The same parameters can also be accessed in the Bio-Rad preprogrammed protocols under LIST > BIO-RAD > MIXED MW.

1. Turn on the Trans-Blot Turbo system using the switch located on the right side of the instrument. After an initial Boot screen, the system will proceed to the Home screen.
2. On the Home screen press the Navigation button that corresponds with Turbo (Figure 12).

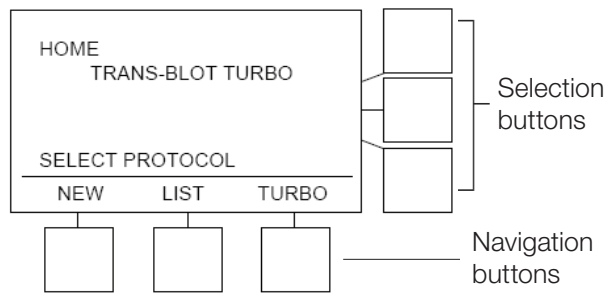


Fig. 12. Home Screen of the Trans-Blot Turbo system.

3. After selecting Turbo, there will be a selection screen for the number, size, and type of gel to be transferred. Use the corresponding selection button to choose the option for the combination of gels in the run. The option varies the current for the run (1.3 A for a single mini format gel, 2.5 A for a single midi or 2 mini format gels). Mini PROTEAN® TGX™ gels can be transferred using the “1 Mini Gel” protocol or the “1 Mini PROTEAN TGX” protocol, which transfers a single Mini PROTEAN TGX gel in 3 min.
4. Press the Navigation button that corresponds to A:RUN for the cassette in the upper bay or B:RUN for the cassette in the lower bay (Figure 13). A beep will sound to signal the start of the transfer for the chosen cassette. If you are running both cassettes, press the button to begin one transfer (either A:RUN or B:RUN), and then press the other button at any time during the run to immediately start the second cassette. Both cassettes must use the same protocol.

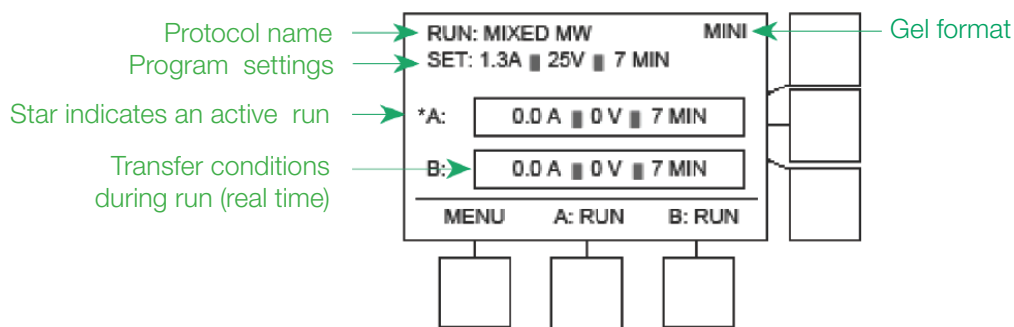


Fig. 13. Transfer Turbo Run screen.

5. The protocol will run automatically. The screen will display the conditions of the transfer and the progress of the run.

Note: A run can be paused by pressing the corresponding A: STOP or B: STOP Navigation button during the run. The user has a choice to continue from the time point of the pause, restart the run from the beginning, or terminate the run (Figure 14.)

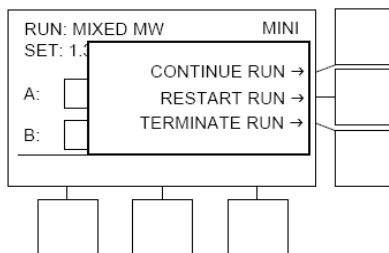


Fig. 14. Trans-Blot Turbo system Terminate Run screen.

When the transfer protocol is complete, the screen will display RUN COMPLETE, and an alarm will be heard (Figure 15).

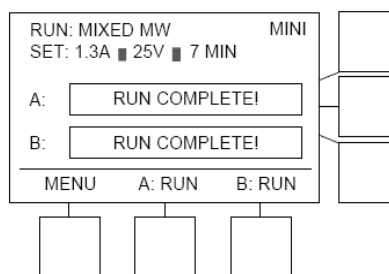


Fig. 15. Trans-Blot Turbo Run Complete screen.

3.4.a. Disassembling and Removing the Membrane

Note: Use caution when removing a cassette from the unit after a transfer run. The cassette may be warm.

1. Remove the cassette from the bay by pulling it straight out of the instrument. The LCD menu screen will automatically return to the protocol screen that has just been completed. The system is ready for another run. If a different protocol or combination of gels will be blotted in the next run, refer to Table 1 (page 7) for the appropriate combinations of gels that can be used in a single run, and proceed to the Home menu to select the correct protocol.
2. Unlock the cassette by turning the dial counterclockwise to the Unlock position.
3. Disassemble the blotting sandwich and place the membrane in a suitable container. If you are using a PVDF membrane, place it immediately into a storage solution (for example, deionized water or blocking or staining solution) as the membrane will quickly dry out. If a PVDF membrane requires rewetting, dip it in methanol or ethanol until uniformly opaque, then wash with deionized water.
4. Discard the ion reservoir stacks after one use; do not attempt to reuse them.
5. Empty residual liquid from the cassette. If no additional transfer will be performed immediately, rinse the base and lid of the cassette with deionized water and dry them with a paper towel.
6. Turn off the Trans-Blot Turbo system with the power switch if it is no longer required.

3.5 Transfer Using Preprogrammed Protocols

All steps are identical to those in Sections 3.3 and 3.3.a, except for Section 3.3, step 2, which is described here.

On the Home screen press the Navigation button that corresponds with LIST (Figure 12) to choose between the Bio-Rad preprogrammed protocols and user-defined protocol lists (Figure 16).

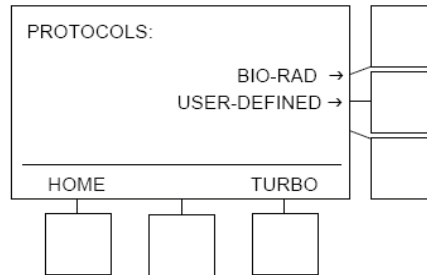


Fig. 16. Protocol options.

Pressing the Bio-Rad preprogrammed protocols button accesses the protocols described in Table 3.

- **STANDARD SD** provides typical semi-dry transfer conditions for use with conventional semi-dry western blotting consumables (see Section 3.6)
- **1.5 mm GEL** uses a longer transfer time (10 min) for more efficient transfer when using 1.5 mm thick gels
- **HIGH MW** is optimized for more efficient transfer of large proteins (>150 kDa)
- **LOW MW** is optimized for more efficient transfer of small proteins (<30 kDa)
- **MIXED MW** is for efficient transfer of proteins over a broad range of molecular weights (5–150 kDa). This protocol is also accessed via the Turbo Navigation button
- **Mini-TGX** is an ultrafast protocol that will transfer a single Mini-PROTEAN TGX gel with mixed MW proteins (5–150 kDa) in 3 min with excellent efficiency.

Table 3. Bio-Rad preprogrammed protocols.

Protocol Name	MW (kDa)	Time (min)	2 Mini Format Gels or 1 Midi Format Gel (per cassette)	1 Mini Format Gel (per cassette)
Standard SD	Any	30	Up to 1.0 A; 25 V	
1.5 mm GEL	Any	10	2.5 A; up to 25 V	1.3 A; up to 25 V
High MW	>150	10		
Low MW	<30	5		
Mixed MW (Turbo)	5–150	7		
1 Mini-TGX	5–150	3	–	2.5 A; up to 25.0 V

Selecting USER-DEFINED protocols accesses the protocols that have been previously saved by users (Figure 17). Use the top and bottom Selection buttons to scroll to the appropriate choice. Push RUN to go to the system run screen where a transfer can be started.

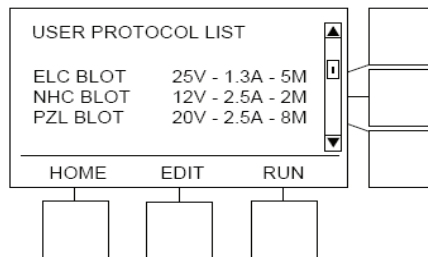


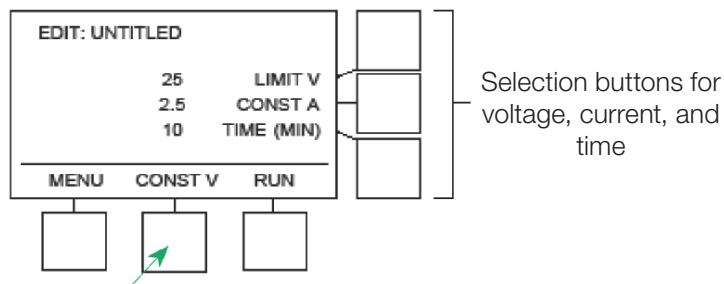
Fig. 17. User protocols screen.

3.6 Transfer Using a New Protocol

Assembly of the blotting sandwich is described in sections 3.1 and 3.2. This section describes setting up a new protocol.

On the Home screen (Figure 12), press the navigation button that corresponds with NEW.

Pressing NEW will bring up the Edit screen (Figure 18), where the user can set the three parameters of current (maximum 2.5 A), voltage (maximum 25 V), and time (maximum 90 min) using the alphanumeric keypad. The user can toggle between setting a constant voltage (CONST V) or a constant current (CONST A) using a navigation button. After the settings have been determined, a prompt will appear to name and save the protocol for subsequent use, if desired. A saved protocol can later be accessed under LIST > USER-DEFINED protocols. Saved protocols can be deleted when no longer required.



Toggle between CONST A and CONST V

Fig. 18. Edit NEW protocol screen.

To prevent damage to the cassette or instrument, the Trans-Blot Turbo system will terminate a transfer if 30 watts per hour is exceeded over the course of the run. Depending on the initial run conditions when designing a protocol, the system may display a message warning that the 30 watts/hour limit may be exceeded during the run.

3.7 Transfer Using Traditional Semi-Dry Consumables

Typical procedure utilizing the Trans-Blot Turbo system and conventional semi-dry western blotting consumables is detailed below:

1. Equilibrate the gel in Towbin transfer buffer (25 mM Tris, 192 mM glycine pH 8.3, 20% MeOH) for 10 min.
2. Soak two pieces of extra-thick (2.4 mm) filter paper in transfer buffer. Six pieces of thick (0.8 mm) filter paper can be used if extra-thick paper is not available.
3. While the gel is equilibrating, prepare a transfer membrane. Wet a nitrocellulose membrane briefly in transfer buffer or PVDF membrane in methanol or ethanol for 30 sec, then wash in water for 1–2 min, and equilibrate in transfer buffer for at least 10 min with agitation.
4. Assemble the transfer sandwich on the cassette base (anode) by placing one piece of wet extra-thick or 3 pieces of thick filter paper on the bottom, then the membrane, the gel, and finally, the remainder of the wet filter paper on top. Use the blot roller to remove air from between the assembled layers (Figure 19).
5. Once the stacks are positioned in the cassette base, place the cassette lid on the base. The lid is reversible, but ensure that the electrical contacts fit closely into the slots in the base. Press the lid down firmly and turn the dial clockwise to engage the lid pins into the locking slots.
6. Load a second cassette if desired. Refer to Table 1 for the appropriate combinations of gels that can be combined in a single run.
7. Slide the cassette (with the dial facing up) into the bay until it makes contact with the magnetic interlock and you hear a click. Cassettes can be inserted into the bays in any order, with or without power to the system.
8. Select the LIST button from the Home menu and the **STANDARD SD** transfer protocol from the Bio-Rad preprogrammed protocols or the user-defined protocol of choice.
9. To initiate the run, press the navigation button that corresponds to A:RUN for the cassette in the upper bay or B:RUN for the cassette in the lower bay.

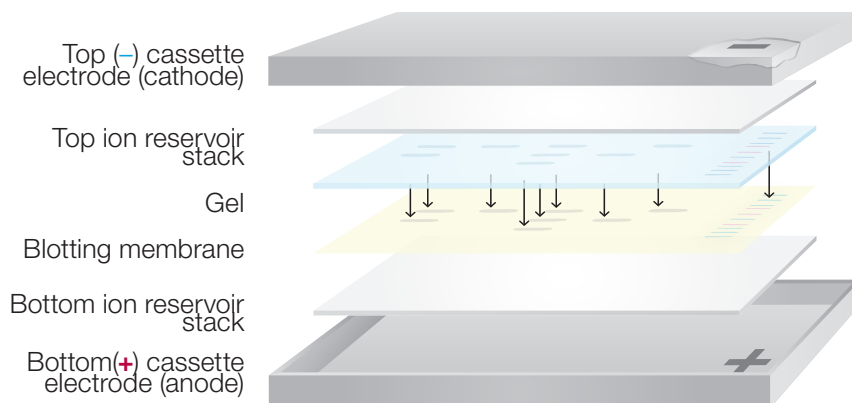


Fig. 19. Proper assembly of blotting sandwich using traditional consumables.

For further information, refer to the Bio-Rad Protein Blotting Guide: A Guide to Protein Detection and Blotting, bulletin 2895, for detailed information. Bulletin 2895 can be downloaded from our website, www.bio-rad.com, as a PDF file, or contact Technical Support at 1-800-424-6723.

3.8 Optimizing Transfer Conditions

The following techniques, alone or in combination, will increase transfer efficiency:

- Use a low-percentage gel or a gradient gel. High-percentage gels retard protein transfer, especially with large proteins
- High molecular weight proteins may require increased transfer times, particularly when using thick gels. Increase the transfer time or power conditions
- With long transfer times or high power conditions, some very low molecular weight proteins may transfer through the membrane to the lower ion reservoir stack. Use a shorter transfer time or reduce power conditions for most efficient transfers
- Use the blot roller to remove any air bubbles when assembling the transfer sandwich. Air bubbles between layers of the assembled sandwich will prevent protein transfer, producing blank spots on the membrane.

Table 4. Recommended power conditions for transfer using the Trans-Blot Turbo system.

	Single Mini Gel	Two Mini Gels or One Midi Gel
With Turbo Transfer Packs	25 V, 1.3 A, 7 min	25 V, 2.5 A, 7 min
With Standard Semi Dry Components	25 V, 1.0 A, 30 min	

Refer to the Bio-Rad Protein Blotting Guide, bulletin 2895, for more information on optimizing electrophoretic transfer. The Protein Blotting Guide can be downloaded from our website, www.bio-rad.com, as a PDF file, or call Technical Support at 1-800-424-6723.

Section 4: Appendices

Appendix A. Maintenance

The Trans-Blot® Turbo™ system requires little maintenance. After you finish using the Trans-Blot Turbo system, rinse each cassette, base, and lid in deionized water to remove residual salts and to prevent salt buildup. Air-dry or use a paper towel to dry the cassette.

For each transfer cassette there are four connection pins located at the back of the instrument base cavity (Figure 20). Over time it is possible that these pins have been exposed to buffer or other contaminants causing them to stick in the depressed position. With the unit unplugged, use isopropyl alcohol and a cotton swab or isopropyl wipes to clean these pins. Push on each pin to ensure that it depresses freely. The pins should be checked and cleaned monthly as part of standard equipment maintenance procedures.

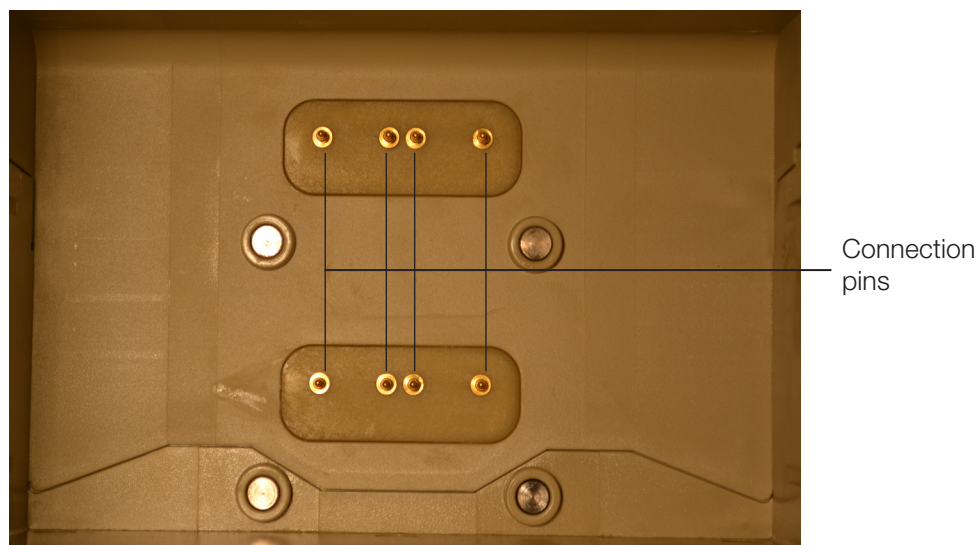


Fig. 20. Connection pins inside the instrument base cavity.

Clean any accidental buffer spills in the instrument bays with a dry towel.

The cassettes and instrument casing can be cleaned with water and mild detergent; do not use abrasives or organic solvents.

Periodically unplug the instrument and wipe the instrument casing with a moist cloth or paper towel. Ensure that the electrode contacts in the bays are clean. Electrode plates should be occasionally cleaned with deionized water or a mild detergent to reduce salt buildup. If the cathode plate has excessive salt buildup, 7% acetic acid can be used to remove the residue.

Occasionally check that the cooling fan vent is free of debris and dust.

Appendix B. Troubleshooting

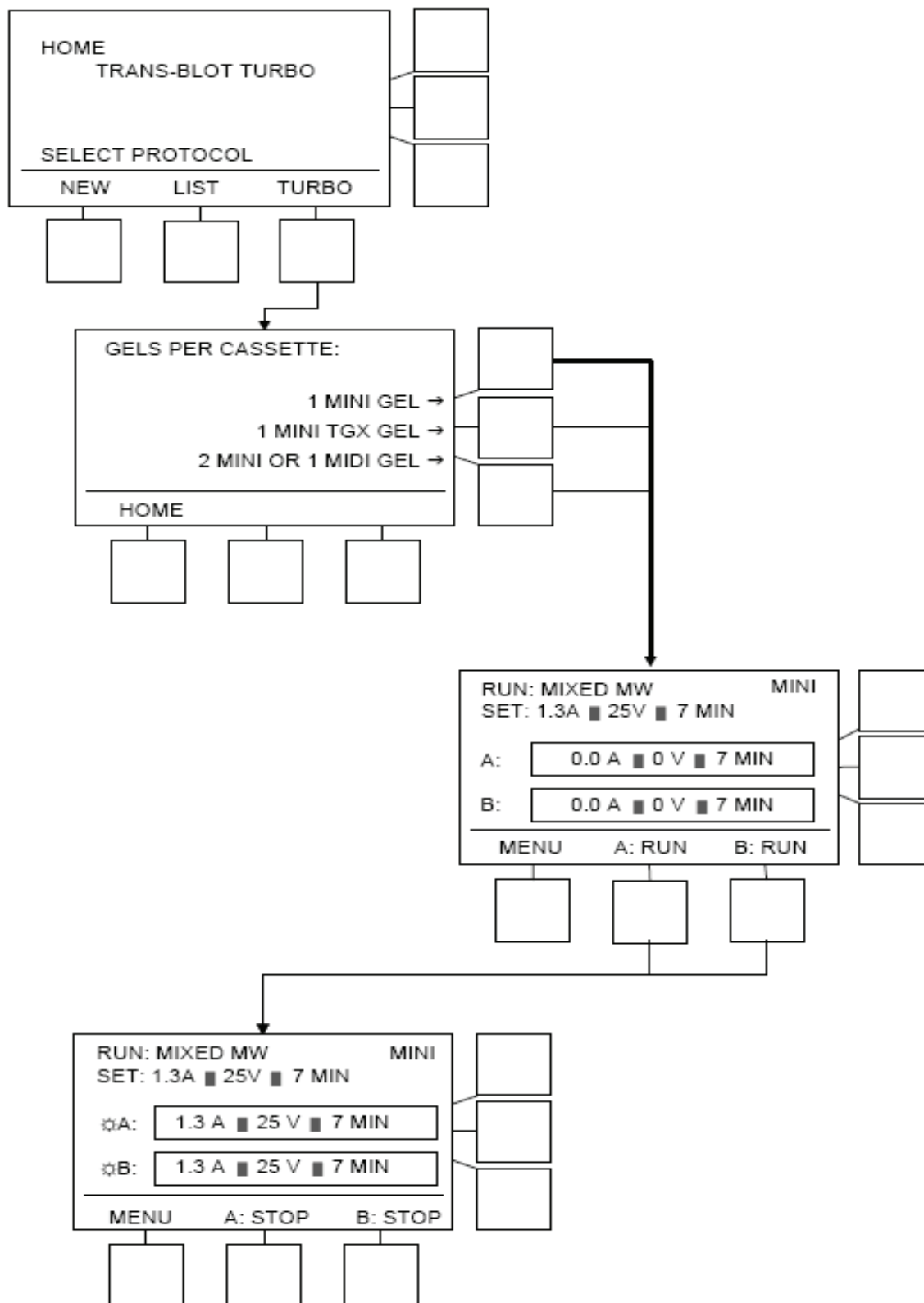
For more information on troubleshooting, see the Bio-Rad Protein Blotting Guide: A Guide to Protein Detection and Blotting, bulletin 2895. This can be downloaded from our website, www.bio-rad.com, as a PDF file, or call Technical Support at 1-800-424-6723.

Symptom/Problem	Suggested Corrections
Poor or no electrophoretic transfer; excessive protein remains in the gel (as detected by staining the gel after transfer)	<p>Transfer time may be too short. High molecular weight proteins (>150 kDa) may require increased transfer time.</p> <p>Change the percentages of the components for handcast gels. The Bio-Rad Protein Blotting Guide: A Guide to Protein Detection and Blotting, bulletin 2895, has extensive information on casting acrylamide gels.</p> <p>The wrong protocol relative to the gel combination was used for the transfer. Refer to Table 1 for acceptable combinations of gels that can be combined in a single run.</p> <p>There may be an excessive amount of protein loaded on the gel. Reduce the amount of protein on the gel.</p> <p>While the preprogrammed protocols efficiently transfer most proteins, some proteins may require further optimization. Increase the transfer time in 1 min increments. Change voltage and current if required.</p>
Poor or no electrophoretic transfer; little or no protein remains in the gel (as detected by staining the gel after transfer)	<p>The blotting sandwich may be assembled in the wrong order. See Figure 8 for the correct assembly.</p> <p>The detection system is not working or is not sensitive enough. Include proper antigen controls to test detection kit sensitivity or use a total protein stain or Criterion Stain Free™ system to detect protein transfer to the membrane prior to immunodetection.</p>
Blot sandwich exceeds 70°C after transfer	<p>An incorrect program for the gel combination was used. Refer to Table 1 for the acceptable combinations of gels that can be combined in a single run.</p> <p>Reduce run current, voltage, or time.</p>
Cassette does not stay in place in the instrument bay	<p>Make sure that the dial of the cassette lid is in the Lock position and the lid pins are engaged. The dial should be facing up when the cassette is inserted into the bay.</p> <p>The magnetic interlock may be damaged or have failed. Contact Technical Support for assistance.</p>
POWER FAILURE! PRESS ANY KEY TO CONTINUE is displayed on the screen	<p>Power to the instrument was interrupted during a run. The run is aborted; it will need to be restarted. Shut off the power switch, correct the power outage, and restart the instrument.</p>

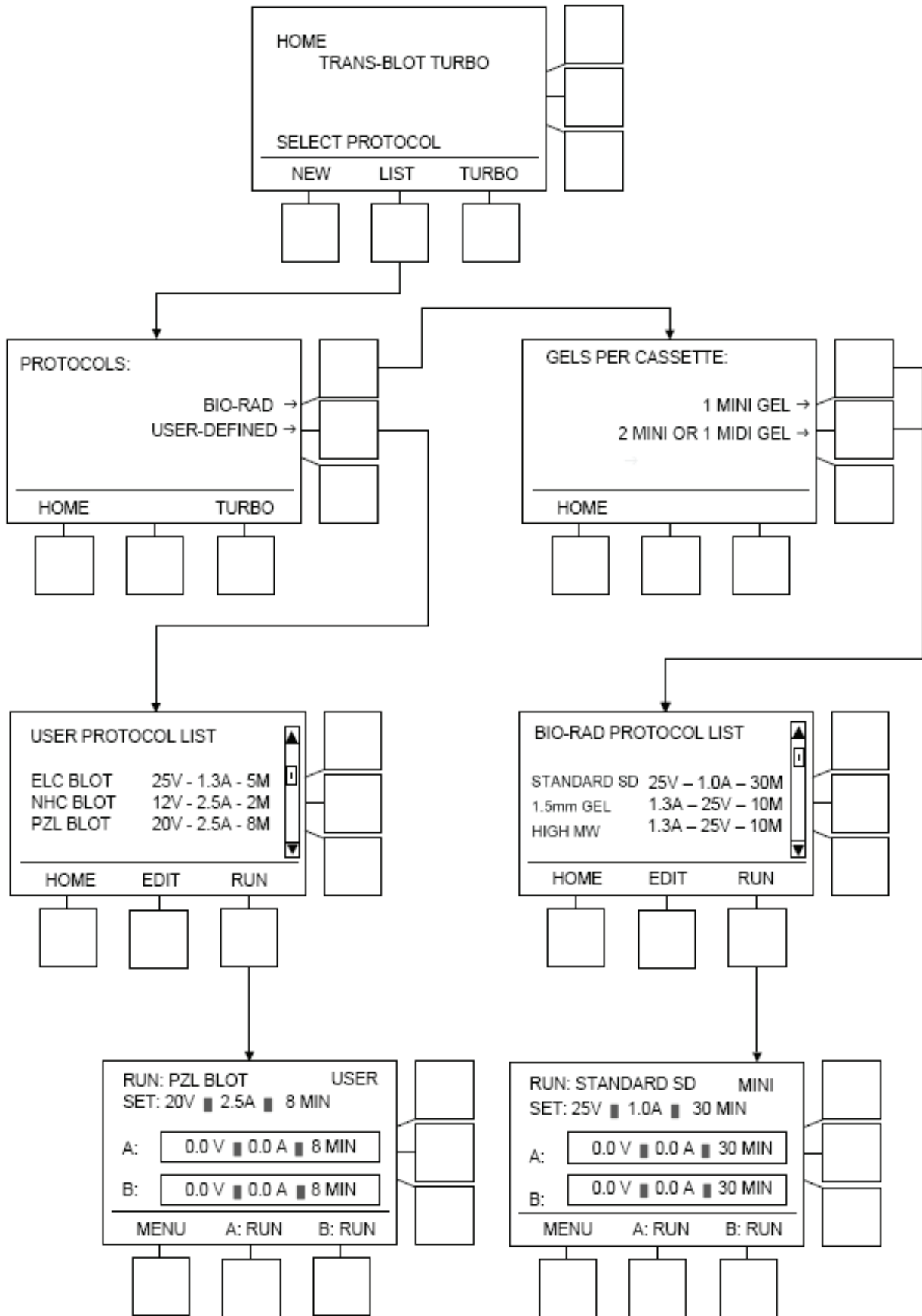
<p>Swirls or missing bands; diffuse transfers</p>	<p>Poor contact between the membrane and the gel. Air bubbles (or excess buffer when using traditional semi-dry consumables) remain between the membrane and gel. Use the blot roller to ensure that air bubbles or excess buffer are removed.</p> <p>Poor contact between the assembled sandwich and the electrodes (when conventional semi-dry consumables are used). Use extra-thick filter paper or add additional sheets of thick buffer-wetted filter paper to improve contact.</p> <p>The membrane was allowed to dry out. White spots on a nitrocellulose or PVDF membrane indicate dry areas where protein will not bind.</p> <p>Power conditions are too high for your particular gel. Adjust voltage and current conditions.</p> <p>The gel electrophoresis may be at fault. Artifacts of electrophoresis may be produced by poor polymerization, inappropriate running conditions, contaminated buffers, sample overload, etc. Consult your electrophoresis manual for more details.</p> <p>When using a single mini transfer pack, the assembled transfer pack may have been placed at the extreme edge of the cassette bottom. Ensure that assembled transfer packs are placed near the center of the cassette base.</p> <p>When two mini gels are transferred simultaneously on a midi format stack, ensure the gels are arranged foot-to-foot (low-molecular weight portions of the gels are towards each other).</p>
<p>NO LOAD DETECTED is displayed on the Run screen</p>	<p>The unit is detecting the presence of a cassette, but current is not passing between the electrode plates.</p> <p>Ensure that the blotting assembly is positioned properly and is thick enough to contact both the anode and cathode plates.</p> <p>If using a custom buffer, the buffer may have an ion capacity that is too low or too high for efficient transfer. Check the concentrations of components in the buffer.</p> <p>The electrical contacts on the cassette or instrument may be dirty. With the instrument unplugged, clean the contacts with a cotton swab or wipe moistened with isopropyl alcohol.</p>

Overview of the Three Protocol Modes

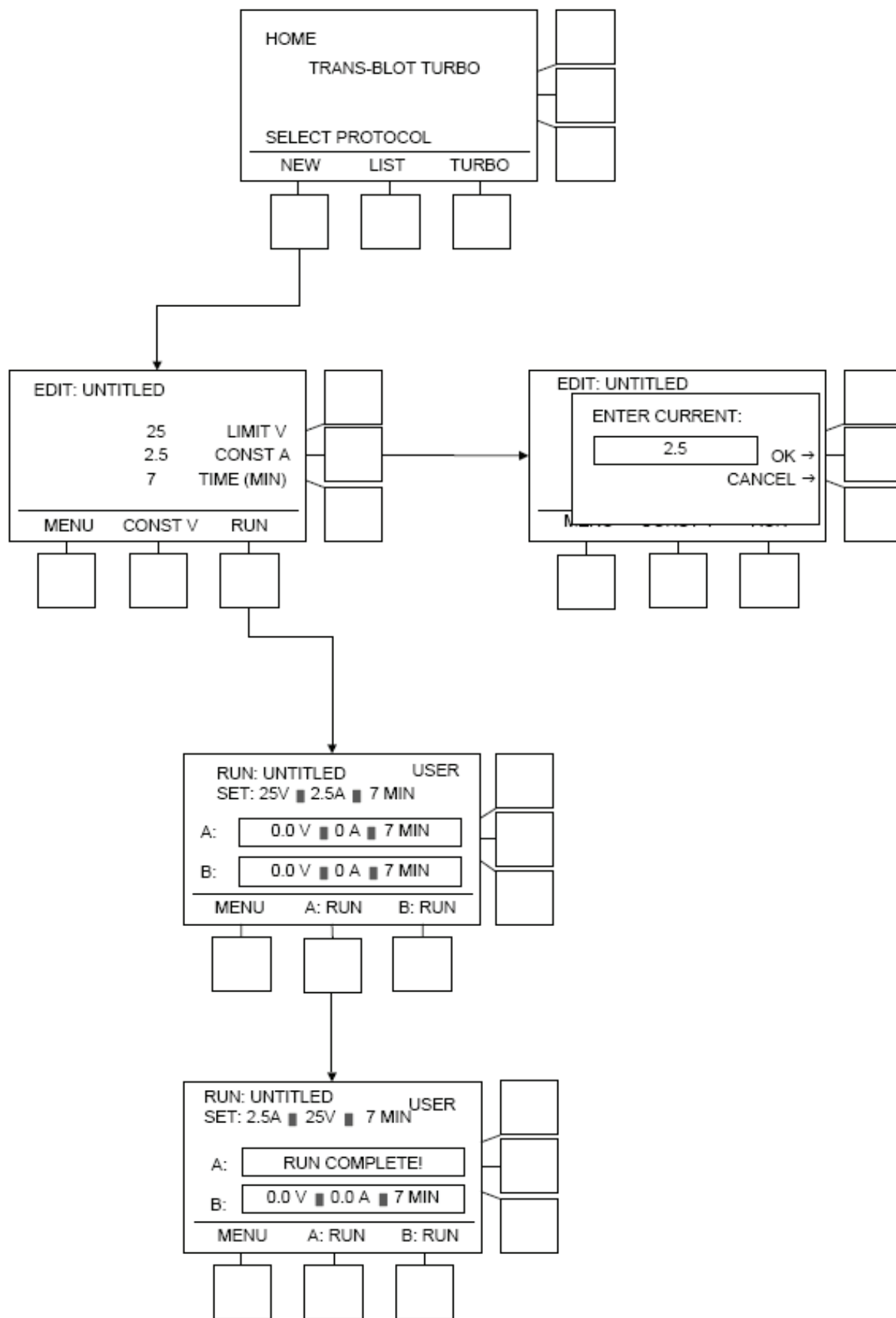
TURBO: 1 mini format or 2 mini format gels, or 1 midi format gel in 7 min; 1 Mini-PROTEAN® TGX™ gel in 3 min



LIST: Bio-Rad preprogrammed protocols or user-defined protocols



NEW: User can edit protocol parameters and name, save, and run the protocol



Consumables and Related Products

Trans-Blot Accessories

170-4151	Trans-Blot® Turbo™ Cassette, single
170-4152	Trans-Blot Turbo Base

Trans-Blot Transfer Packs

170-4155	Trans-Blot Starter Kit, Trans-Blot system, Precision Plus Protein™ WesternC™ standard, and mixed transfer pack sample
170-4156	Trans-Blot Transfer Pack, 0.2 µm PVDF membrane, mini format, pack of 10
170-4157	Trans-Blot Transfer Pack, 0.2 µm PVDF membrane, midi format, pack of 10
170-4158	Trans-Blot Transfer Pack, 0.2 µm nitrocellulose membrane, mini format, pack of 10
170-4159	Trans-Blot Transfer Pack, 0.2 µm nitrocellulose membrane, midi format, pack of 10

Trans-Blot Turbo RTA Transfer Kits

170-4270	Trans-Blot Turbo RTA Transfer Kit, nitrocellulose, mini format (7.0 x 8.5 cm), for 40 blots
170-4271	Trans-Blot Turbo RTA Transfer Kit, nitrocellulose, midi format (13.5 x 8.5 cm), for 40 blots
170-4273	Trans-Blot Turbo RTA Transfer Kit, PVDF, mini format (7.0 x 8.5 cm), for 40 blots
170-4274	Trans-Blot Turbo RTA Transfer Kit, PVDF, midi format (13.5 x 8.5cm), for 40 blots
170-4275	Trans-Blot Turbo RTA Transfer Kit, LF PVDF, mini format (7.0 x 8.5 cm), for 40 blots
170-4276	Trans-Blot Turbo RTA Transfer Kit, LF PVDF, midi format (13.5 x 8.5 cm), for 40 blots

Related Products

170-5070	Immun-Star™ WesternC™ Chemiluminescence Kit, includes 50 ml luminol/enhancer; 50 ml stable peroxide buffer; designed for CCD imaging of western blots
170-5044	Immun-Star GAM-HRP Detection Kit, includes substrate, GAM antibody, blocker, Tween-20, TBS; designed for film detection of western blots
170-5045	Immun-Star GAR-HRP Detection Kit, includes substrate, GAR antibody, blocker, Tween-20, TBS; designed for film detection of western blots
170-5010	Immun-Star GAM-AP Detection Kit, includes substrate, enhancer, GAM antibody
170-5011	Immun-Star GAR-AP Detection Kit, includes substrate, enhancer, GAR antibody
170-8238	Amplified Opti-4CN™ Substrate Kit, includes substrate, diluent, blocker, PBS
170-6515	Blotting-Grade Conjugates, goat anti-rabbit IgG (H+L)-HRP, 2 ml
170-6516	Blotting-Grade Conjugates, goat anti-mouse IgG (H+L)-HRP, 2 ml
170-6404	Blotting-Grade Blocker, nonfat dry milk, 300 g



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