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Trans-Blot® SD Semi-Dry Electrophoretic Transfer Cell

Instruction Manual

Catalog Number

170-3940

BIO-RAD

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Note

To insure the best performance from the Trans-Blot SD semi-dry electrophoretic transfer cell, become fully acquainted with these operating instructions before using the cell to transfer samples. Bio-Rad recommends that you first read these instructions carefully. Then assemble and disassemble the cell completely without transferring sample. After these preliminary steps, you should be ready to transfer a sample.

Bio-Rad also recommends that all Trans-Blot SD cell components and accessories be cleaned with a suitable laboratory cleaner (such as Bio-Rad Cleaning Concentrate, catalog number 161-0722) and rinsed thoroughly with distilled water, before use.

Model_____

Catalog Number_____

Date of Delivery_____

Warranty Period_____

Serial Number_____

Invoice Number_____

Purchase Order Number_____

Warranty

Bio-Rad Laboratories warrants the Trans-Blot SD semi-dry electrophoretic transfer cell against defects in materials and workmanship for 1 year. If any defects occur in the instrument during this warranty period, Bio-Rad Laboratories will repair or replace the defective parts free. The following defects, however, are specifically excluded:

1. Defects caused by improper operation.
2. Repair or modification done by anyone other than Bio-Rad Laboratories or an authorized agent.
3. Use of fittings or other spare parts supplied by anyone other than Bio-Rad Laboratories.
4. Damage caused by accident or misuse.
5. Damage caused by disaster.
6. Corrosion due to use of improper solvent or sample.

This warranty does not apply to parts listed below:

1. Platinum plate electrode.

For any inquiry or request for repair service, contact Bio-Rad Laboratories after confirming the model and serial number of your instrument.

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Section 1
Introduction

Blotting was first performed by Southern[1] in 1975 with the transfer of DNA from agarose gels to nitrocellulose membranes. Blotting has subsequently been applied to RNA[2-4] and protein[5,6] from both agarose and polyacrylamide gels. Membrane materials have been expanded to include PVDF for improved protein binding capacity. To overcome the inefficiency of capillary transfers, electric current has been adopted for eluting proteins from polyacrylamide gels, as first described by Towbin et al.[7] in 1979. Since that time, electrophoretic transfer has also been used for DNA and RNA blotting.[8-14]

For blotting PCR fragments, plasmid and vector DNA, and RNA with the SD cell, use the Trans-Blot SD DNA blotting kit. DNA or RNA can be blotted from agarose gel to Zeta-Probe® GT membrane in only 10 minutes, without any gel pretreatments. The kit comes complete with DNA/RNA blotting accessories and a detailed instruction manual.

Semi-dry blotting was first reported by Kyhse-Andersen in 1984.[15] Blotting was performed with plate electrodes in a horizontal configuration. The gel and nitrocellulose membrane were sandwiched between sheets of buffer-soaked filter paper, which served as the ion reservoir and replaced the buffer tank. The plate electrodes, separated only by the filter paper stack, provided high field strength (V/cm) across the gel, and very efficient, rapid transfers.

The Trans-Blot semi-dry transfer cell incorporates the original concepts of semi-dry blotting along with innovative features for quick set-up and ease of use. The platinum-coated titanium and stainless steel electrode pair provides efficient, background-free blotting with trouble-free service.

1.1 Specifications

Construction	
Trans-Blot SD body	Molded polycarbonate
Anode	Platinum-coated titanium
Cathode	Stainless steel
Anode platform	Precision machined acrylic
Overall size	37 cm x 24 cm x 11 cm
Maximum gel size	25 cm x 18.5 cm
Cleaning	Do not immerse the unit in liquid. Use special care when cleaning the anode plate to avoid scratching or marring the platinum. Do not use abrasives or strong detergents. The cathode plate (stainless steel) can be cleaned with a mild abrasive to remove salt that may deposit during normal operation. The entire unit can also be periodically disassembled and cleaned with water to remove salt deposits.
Chemical compatibility	The semi-dry blotter components are not compatible with chlorinated hydrocarbons (e.g., chloroform), aromatic hydrocarbons (e.g., toluene, benzene), or acetone. Use of organic solvents voids all warranties.

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Section 2
Equipment and Reagents

2.1 Equipment and Accessories

Catalog Number	Product Description
170-3940	**Trans-Blot SD Electrophoretic Transfer Cell**
Replacement Parts	
170-3942	**Trans-Blot SD Anode,** platinum
170-3947	**Trans-Blot SD Cathode,** stainless steel
DNA Blotting Kit	
170-3957	**Trans-Blot SD DNA/RNA Blotting Kit**
Power Supply	
165-4761	**Model 200/2.0 Constant Voltage Power Supply,** 100/120 V, 50/60 Hz
165-4762	**Model 200/2.0 Constant Voltage Power Supply,** 220/240 V, 50/60 Hz

Protein Blotting Accesories

Blot Absorbent Filter Paper (extra thick)		Recommended uses
170-3966	**Sheets,** 7 x 8.4 cm pre-cut to fit mini PROTEAN 3 Gels, 60	Protein blotting
170-3967	**Sheets,** 8.3 x 13.6 cm pre-cut to fit Criterion Gels, 60	
170-3968	**Sheets,** 14 x 16 cm pre-cut to fit PROTEAN xi Gels, 30	
170-3969	**Sheets,** 19 x 19.5 cm pre-cut to fit PROTEAN xL Gels, 30	
Immuno Blot PVDF Membrane		
162-0174	**Sheets,** 7 x 8.4 cm, 10 (mini blot size)	Immunoblotting of protein. High capacity for all molecular weight ranges. Low background for immunodensity.
162-0175	**Sheets,** 10 x 15 cm, 10	
162-0176	**Sheets,** 20 x 20 cm, 10	
162-0177	**Roll,** 26 cm x 3.3 m, 1	
Sequi-Blot PVDF Membrane		
162-0180	**Sheets,** 10 x 15 cm, 10	Protein sequencing and amino acid analysis.
162-0181	**Sheets,** 15 x 15 cm, 10	
162-0182	**Sheets,** 20 x 20 cm, 10	
162-0184	**Roll,** 24 cm x 3.3 m, 1	
162-0185	**Sheets,** 20 x 20 cm, 3	
162-0186	**Sheets,** 7 x 8. 4 cm, 10	
Nitrocellulose Membrane (0.45 micron)		
162-0115	**Roll,** 33 cm x 3 m, 1	Transfer of proteins (high molecular weight or abundant, low molecular weight tend to penetrate this membrane)
162-0113	**Sheets,** 20 x 20 cm, 5	
162-0114	**Sheets,** 15 x 9.2 cm, 10	
162-0116	**Sheets,** 15 x 15 cm, 10	
162-0117	**Sheets,** 9 x 12 cm, 10	
162-0145	**Sheets,** 7 x 8.4 cm, 10	

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Catalog Number	Product Description	
Nitrocellulose Membrane (0.2 micron)		**Recommended uses**
162-0112	**Roll,** 33 cm x 3 m, 1	Transfer of proteins (smaller pore size retaining more low molecular weight proteins - PVDF is even more efficient.
162-0146	**Sheets,** 7 x 8.4, 10	
162-0147	**Sheets,** 13.5 x 16.5 cm, 10	
DNA/RNA Blotting Accessories (Blot paper)		
170-3958	**Extra Thick Blot Paper,** 10 x 15 cm, 30	Nucleic Acid Blotting
170-3959	**Extra Thick Blot Paper,** 15 x 15 cm, 30	
170-3960	**Extra Thick Blot Paper,** 15 x 20 cm, 30	
Zeta-Probe Membrane		
162-0159	**Roll,** 30 cm x 3.3 m, 1	Transfer of single-stranded or double stranded DNA or RNA of all sizes.
162-0153	**Sheets,** 9 x 12 cm, 15	
162-0154	**Sheets,** 10 x 15 cm, 15	
162-0155	**Sheets,** 15 x 15 cm, 15	
162-0156	**Sheets,** 15 x 20 cm, 15	
162-0157	**Sheets,** 20 x 20 cm, 15	
162-0158	**Sheets,** 20 x 25 cm, 3	
162-0165	**Roll,** 20 cm x 3.3 m, 1	
162-0166	**Roll,** 30 cm x 30 m, 1	
Zeta-Probe GT Membrane		
162-0190	**Sheets,** 9 x 12 cm, 15	Transfer of single-stranded and double stranded DNA or RNA of all sizes.
162-0191	**Sheets,** 10 x 15 cm, 15	
162-0192	**Sheets,** 15 x 15 cm, 15	
162-0193	**Sheets,** 15 x 20 cm, 15	
162-0194	**Sheets,** 20 x 20 cm, 15	
162-0195	**Sheets,** 20 x 25 cm, 15	
162-0196	**Roll,** 30 cm x 3.3 m, 1	
162-0197	**Roll,** 20 cm x 3.3 m, 1	
162-0198	**Roll,** 30 cm x 30 m, 1	
Supported Nitrocellulose Membrane (0.45 micron)		
162-0090	**Sheets,** 7 x 8.4 cm, 10	
162-0091	**Sheets,** 10 x 15 cm, 10	
162-0092	**Sheets,** 15 x 15 cm, 10	
162-0093	**Sheets,** 20 x 20 cm, 10	
162-0094	**Roll,** 30 cm x 3 m, 1	
Supported Nitrocellulose Membrane (0.2 micron)		
162-0095	**Sheets,** 7 x 8.4 cm, 10	
162-0096	**Sheets,** 15 x 15 cm, 10	
162-0097	**Roll,** 30 cm x 3 m, 1	

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2.2 Related Instruments

Catalog Number	Product Description
Blotting Equipment	
170-3910	**Trans-Blot Electrophoretic Transfer Cell**
170-3946	**Trans-Blot Electrophoretic Transfer Cell,** with plate electrodes
170-3945	**Trans-Blot Plate Electrode Pair**
170-3930	**Mini Trans-Blot® Electrophoretic Transfer Cell**
170-3970	**Western Processor**
170-6545	**Bio-Dot® Microfiltration Apparatus**
170-6542	**Bio-Dot SF Microfiltration Apparatus**
170-4017	**Mini-PROTEAN® II Multiscreen Apparatus**
170-4037	**Large Incubation Tray**
170-4039	**Large Incubation Tray Lid**
170-4041	**Small Incubation Tray**
170-4045	**Small Incubation Tray Lid**

2.3 Chemical Reagents

Immun-Blot® Assay Kits

Immun-Blot assay kits contain the necessary components and instructions for performing immune detection assays on blotted membranes.

Catalog Number	Product Description
Immun-Blot Assay Kits	
170-6460	**Immun-Blot Assay Kit** - Goat Anti-Rabbit AP
170-6461	**Immun-Blot Assay Kit** - Goat Anti-Mouse AP
170-6462	**Immun-Blot Assay Kit** - Goat Anti-Human AP
170-6463	**Immun-Blot Assay Kit** - Goat Anti-Rabbit HRP
170-6464	**Immun-Blot Assay Kit** - Goat Anti-Mouse HRP
170-6465	**Immun-Blot Assay Kit** - Goat Anti-Human HRP
170-6466	**Immun-Blot Assay Kit** - Protein A-HRP
170-6467	**Immun-Blot Assay Kit** - Protein G-HRP
Immun-Lite™ Chemiluminescent Assay Kits, include Immun-Lite Membrane, 10 x 15 cm, 15 sheets	
170-6470	**Immun-Lite Assay Kit,** Goat Anti-Rabbit AP
170-6471	**Immun-Lite Assay Kit,** Goat Anti-Mouse AP
170-6472	**Immun-Lite Assay Kit,** Goat Anti-Human AP
Immun-Lite II Assay Kits, for use with nitrocellulose and PVDF membranes	
170-6477	**Immun-Lite II Assay Kit,** Goat Anti-Rabbit AP
170-6478	**Immun-Lite II Assay Kit,** Goat Anti-Mouse AP
170-6479	**Immun-Lite II Assay Kit,** Goat Anti-Human AP

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Catalog Number	Product Description
Total Protein Detection Kits	
170-6512	**Biotin-Blot Protein Detection Kit**
170-6517	**Enhanced Colloidal Gold Total Protein Detection Kit**
Blotting Standards	
161-0372	**Precision Prestained Standards,** 10–250 kD, 500 µl
161-0380	**Precision Streptactin-HRP conjugate**
161-0381	**Precision Streptactin-AP conjugate**
161-0305	**Prestained SDS-PAGE Standards,** Low range
161-0309	**Prestained SDS-PAGE Standards,** High range
161-0307	**Biotinylated SDS-PAGE Standards Kit,** Low range, HRP
161-0308	**Biotinylated SDS-PAGE Standards Kit,** Low range, AP
161-0312	**Biotinylated SDS-PAGE Standards Kit,** High range, HRP
161-0313	**Biotinylated SDS-PAGE Standards Kit,** High range, AP
161-0306	**Biotinylated SDS-PAGE Standards,** Low range, 250 µl
161-0311	**Biotinylated SDS-PAGE Standards,** High range, 250 µl
Premixed Electrophoresis and Blotting Buffers	
161-0732	**10X Tris/Glycine/SDS,** 1 L
161-0755	**10X Tris/Glycine/SDS,** 6 x 1 L
161-0734	**10X Tris/Glycine,** 1 L
161-0757	**10X Tris/Glycine,** 6 x 1 L
161-0733	**10X Tris/Boric Acid/EDTA,** 1 L
161-0756	**10X Tris/Boric Acid/EDTA,** 6 x 1 L
161-0741	**10X TBE Extended Range,** 1 L
161-0758	**10X TBE Extended Range,** 6 x 1 L

Section 3
Safety Instructions

Read the entire manual before beginning electrophoretic transfers.

Electrophoretic transfer of proteins and nucleic acids is dependent on many factors. Observe the following guidelines to avoid mishaps that may result in serious damage to the instrument or injury to the operator.

1. **Do not reverse polarity on this instrument.** This will result in corrosion and rusting of the stainless steel cathode. If this should occur, the stainless steel should be cleaned with a mild abrasive cleaner to remove the rust.
2. **Do not exceed 25 V with this instrument.** This could damage the electrodes.
3. **Do not adjust the pH of transfer buffers unless specifically indicated.** Follow instructions carefully. Adjustment of pH of transfer buffers, when not indicated, will result in increased buffer conductivity. This is manifested by a higher than expected initial current output as shown by the power supply's current meter. Monitor buffer resistance with the Model 200/2.0 power supply prior to each run to insure proper buffer conductivity.

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4. **Lengthy transfer times are not recommended.** Do not leave this instrument unattended. Joule heat can be generated rapidly during semi-dry blotting. Transferring longer than 2 hours can damage the unit.

5. **Power supply requirements.** The Trans-Blot SD cell should only be used with the microprocessor-controlled Model 200/2.0 power supply (catalog numbers 165-4761 and 165-4762), or the Model 1000/500 power supply (catalog numbers 165-4710 and 165-4711). Do not use the Model 250/2.5 power supply with this apparatus. The low voltage, high current operating conditions of the Trans-Blot SD cell are not compatible with the Model 250/2.5 power supply, and will cause the power supply to blow a fuse.

6. **Do not operate this instrument in ambient temperatures exceeding 50 °C.**

   Important

   This Bio-Rad instrument is designed and certified to meet IEC 1010-1* safety standards. Certified products are safe to use when operated in accordance with the instructtion manual. This instrument should not be modified in any way. Alteration of this instrument will:

   - **Void the manufacturer's warranty**
   - **Void the IEC1010-1 safety certification**
   - **Create a potential safety hazard**

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

   *IEC 1010-1 is an internationally accepted electical safety standard for laboratory instruments.

Section 4
Trans-Blot SD Assembly

To determine the optimum conditions for a particular sample, a time course of transfer should be performed. Since many factors affect transfer *e.g.* molecular weight, pI, and porosity of the gel, transferring for the full suggested time may not be necessary.

4.1 Preparation for Blotting

1. Prepare the transfer buffer. See Section 5 for buffer formulation.

   Note: Buffer preparation is extremely important. **Do not** adjust transfer buffer pH by addition of acid or base unless specifically indicated in the instructions. **Improperly prepared buffer will cause excess heat generation and safety hazards.** Use only high quality, reagent grade methanol. Contaminated methanol can result in increased transfer buffer conductivity, as well as poor transfer of macromolecules.

2. Following electrophoresis, equilibrate the gels in transfer buffer. Equilibration facilitates the removal of electrophoresis buffer salts and detergents. If the salts are not removed, they will increase the conductivity of the transfer buffer and the amount of heat generated during the transfer. Also, low percentage gels (<12% acrylamide) will shrink in methanol-containing buffers. Equilibration allows the gel to adjust to its final size prior to electrophoretic transfer. The length of time required for equilibration is dependent on the gel thickness. For example, 15 minutes for a 0.75 mm SDS-PAGE gel.

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Low molecular weight macromolecules (10,000 daltons) may diffuse out of gels more readily. One can allow adequate gel pre-equilibration by changing the pre-equilibration buffer several times during a relatively short pre-equilibration period. This will help to limit diffusion of low molecular weight macromolecules while providing efficient salt reduction.

3. Cut the membrane to the dimensions of the gel. Wet the membrane by slowly sliding it at a 45° angle into transfer buffer and allowing it to soak for 15–30 minutes. Complete wetting of the membrane is important to insure proper binding. Abrupt wetting can lead to entrapment of air bubbles in the matrix. These air bubbles can block transfer of molecules. To avoid membrane contamination, always use forceps or wear gloves when handling membranes.

4. Cut filter paper to the dimensions of the gel. Two pieces of extra thick filter paper (or four pieces of thick or six pieces of thin filter paper) per gel are needed for each gel/membrane sandwich. Completely saturate the filter paper by soaking in transfer buffer.

5. If more than one full-size gel is to be transferred at one time, cut a piece of dialysis membrane with the appropriate molecular weight cutoff to the dimensions of the gel. Completely wet the dialysis membrane in transfer buffer. Spectr/Por™ dialysis membrane is recommended for this use.

4.2 Assembly of the Unit for Standard Transfers

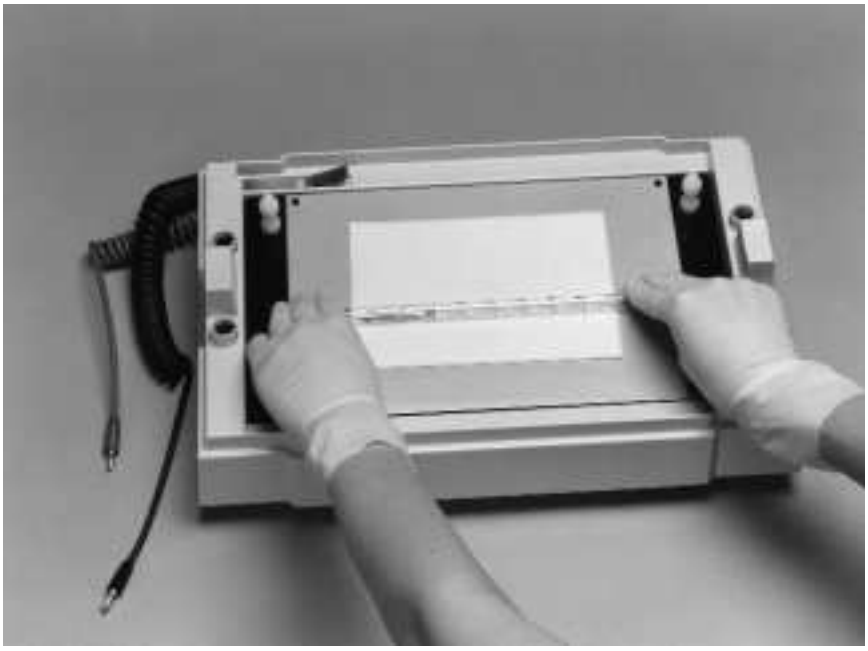
Wear gloves for this procedure to avoid contamination of membranes.

1. Remove the safety cover and the stainless steel cathode assembly.



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2. Place a pre-soaked sheet of extra thick filter paper onto the platinum anode. Roll a pipet or test tube over the surface of the filter paper (like a rolling pin) to exclude all air bubbles. If thick or thin filter paper is used, repeat with one or two more sheets of buffer-soaked filter paper.



3. Place the pre-wetted blotting media on top of the filter paper. Roll out all air bubbles.
4. Carefully place the equilibrated gel on top of the transfer membrane, aligning the gel on the center of the membrane. Transfer will be incomplete if any portion of the gel is outside the blotting media. Roll out all air bubbles.
5. Place the other sheet of pre-soaked filter paper on top of the gel, carefully removing air bubbles from between the gel and filter paper. If thick filter paper is used, place two sheets on top of the gel, and remove bubbles from between each layer. If thin filter paper is used, place three sheets on top of the gel, and remove bubbles from between each layer.
6. If more than one full-size gel is to be transferred, place a sheet of pre-soaked dialysis membrane on top of the filter paper stack. Repeat the procedure from step 2. Up to four mini gels can be transferred at the same time by placing them side-by-side on the anode platform.

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7. Carefully place the cathode onto the stack. Press to engage the latches with the guide posts without disturbing the filter paper stack.



8. Place the safety cover on the unit. Plug the unit into the power supply. Normal transfer polarity is cathode to anode, *i.e.*, red wire to red outlet and black wire to black outlet on the power supply.

   Caution: Do not reverse polarity. This will result in damage to the stainless steel cathode.

9. Turn on the power supply. Transfer mini gels for 15–30 minutes at 10–15 V. Large gels can be transferred for 30 minutes to 1 hour at 15–25 V. Do not exceed 25 V with this instrument. A current limit (3 mA/cm^2 for large gels; 5.5 mA/cm^2 for mini gels) is recommended to prevent excessive heating during the run. Under the strong fields developed by this apparatus, transfers may not always be quantitative. A certain quantity of protein may be transferred through the membrane and onto the filter paper below.

   The Model 200/2.0 power supply is capable of a 200 watt output. This means that unless a current limit is set, uncontrolled conductivity changes may result in full power being delivered to the Trans-Blot SD cell. In this situation, the gel sandwich and electrodes will be exposed to excessive heat. This may result in a safety hazard. It is advisable to monitor resistance, power, and current during the run. Refer to the Model 200/2.0 Instruction Manual for setting current limits and run times, and monitoring these parameters.

10. Following transfer, turn the power supply off, and disconnect the unit from the power supply. Remove the safety cover and the cathode assembly. Discard the filter paper (and dialysis membrane, if used). The transfer efficiency can be monitored by staining the gel with Coomassie blue R-250 protein stain or with Bio-Rad's Silver Stain Kit. Alternatively, prestained molecular weight standards can be used, or a portion of the membrane can be stained for total protein with colloidal gold, Biotin Blot Total Protein Stain, or an anionic dye such as Amido Black. Zeta-Probe membrane can be stained with the Biotin-Blot Total Protein Stain.

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4.3 Assembly of the Unit for Acidic Transfers

If an acidic transfer buffer is used, the transfer direction will be from the anode to the cathode.

1. Remove the safety cover and the stainless steel cathode assembly.
2. Place a pre-soaked sheet of extra thick filter paper onto the platinum anode. Roll out all air bubbles. If thin filter paper is used, repeat with two more sheets of buffer-soaked filter paper. If thick filter paper is used, repeat with one more sheet of buffer soaked filter paper.
3. Carefully place equilibrated gel on top of the filter paper, aligning the gel on the center of the membrane. Roll out all air bubbles.
4. Place the pre-wetted blotting media on top of the gel. Roll out all air bubbles.
5. Place another sheet of pre-soaked extra thick filter paper on top of the blotting membrane, carefully removing all air bubbles. If thin filter paper is used, place three sheets on top of the membrane, or if thick filter paper is used, place two sheets on top of the membrane.
6. If more than one gel is to be transferred, place a sheet of pre-soaked dialysis membrane on top of the filter paper stack. Repeat the procedure from step 2.
7. Carefully place the cathode assembly onto the stack. Press to engage the latches with the guide posts, without disturbing the filter paper stack.
8. Place the safety cover on the unit. Plug the unit into the power supply, red wire to red outlet and black wire to black outlet.

   Caution: Do not reverse polarity. This will damage the stainless steel cathode.
9. Turn on the power supply. Transfer mini gels for 15–30 minutes at 10–15 V. Large gels can be transferred for 30 minutes to 1 hour at 15–25 V. Do not exceed 25 V with this instrument. A current limit (3 mA/cm^2 for large gels; 5.5 mA/cm^2 for mini gels) is recommended to prevent excessive heating during the run.

Section 5
Buffer Formulation

The following buffers are recommended for use with the Trans-Blot SD cell. For protein transfers, the single buffer system of Bjerrum and Schafer-Nielsen[16] provides more efficient elution than the original isotachophoretic system of Khyse-Andersen, which requires the use of three different buffers.[15] A carbonate buffer has also been shown to produce high efficiency transfers with improved antibody recognition.

1. Bjerrum and Schafer-Nielsen transfer buffer for SDS-proteins using nitrocellulose (with methanol) or Zeta-Probe membrane (without methanol):[16]

   48 mM Tris, 39 mM glycine, (20% methanol) pH 9.2
   Dissolve 5.82 g Tris and 2.93 g glycine [and 0.375 g SDS or 3.75 ml of 10% SDS] in dd H_2O (add 200 ml of methanol); adjust volume to 1 liter with dd H_2O.

   DO NOT ADD ACID OR BASE TO ADJUST pH. The buffer will range from pH 9.0 to 9.4, depending on the quality of the Tris, glycine, dd H_2O, and methanol. Methanol should be analytical reagent grade, because metallic contaminants in low grade methanol will plate on the electrodes.

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Note: Some pH electrodes will not perform a proper measurement for the pH of Tris buffers. If the pH of the buffer is not correct, check the electrode to be sure it is designed to function with Tris buffers. If the pH electrode works properly with Tris buffers, and the pH is below 9.0, remake the buffer.

2. SDS may be added to Buffer 1 to increase protein elution from the gel:

   48 mM Tris, 39 mM glycine, (20% methanol), 1.3 mM SDS (0.0375%), pH 9.2
   Dissolve 5.82 g Tris and 2.93 g glycine, and 0.0375 g SDS or 3.75 ml of 10% SDS in dd H_2O (add 200 ml of methanol); adjust the volume to 1 liter with dd H_2O.

   DO NOT ADD ACID OR BASE TO ADJUST pH.

3. Towbin transfer buffer for SDS-proteins using nitrocellulose (with methanol) or Zeta-Probe membrane (without methanol):[7]

   25 mM Tris, 192 mM glycine (20% methanol), pH 8.3
   Dissolve 3.03 g Tris and 14.4 g glycine in dd H_2O (add 200 ml of methanol); adjust volume to 1 liter with dd H_2O.

   DO NOT ADD ACID OR BASE TO ADJUST pH.

4. Dunn carbonate transfer buffer for SDS-proteins using nitrocellulose (with methanol) or Zeta-Probe membrane (without methanol):[17]

   10 mM $NaCHO_3$, 3 mM Na_2CO_3 (20% methanol), pH 9.9
   Dissolve 0.84 g $NaHCO_3$ and 0.318 g Na_2CO_3 (anhydrous) in dd H_2O (add 200 ml of methanol); adjust volume to 1 liter with dd H_2O.

   DO NOT ADD ACID OR BASE TO ADJUST pH.

5. DNA transfer buffer for use with Zeta-Probe membrane:[18]

   5x TBE stock solution (0.5 M Tris, 0.5 M boric acid, 10 mM EDTA in dd H_2O; adjust volume to 1 liter with dd H_2O. Dilute to 0.5x TBE with dd H_2O for the working solution.

   DO NOT ADD ACID OR BASE TO ADJUST pH.

6. 5x dye buffer (20% Ficoll, 20 mM EDTA, 1% SDS, 0.2% bromophenol blue)

Section 6
Examples of Specific Protocols

Note: In order to determine the optimum conditions for a particular sample, a time course of transfer should be performed. Since many factors affect transfer, *e.g.*, molecular weight, pI, porosity of the gel, it may not be necessary to transfer for the full time or to use high field intensity transfer conditions. Final transfer conditions for any protein should be determined empirically.

6.1 SDS-Protein Blotting

Standard Blot to Nitrocellulose

1. Equilibrate the gel in 500 ml of Towbin buffer (Section 5) for 15 minutes.
2. Pre-chill buffer prior to transfer.
3. Assemble the sandwich as described in Section 4.2.
4. Refer to Section 4.2, step 9 for transfer conditions with either large or small gels.

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6.2 DNA Blotting (For acrylamide gels with DNA 250 bp to ~1 kb)

Electrophoresis Run on a Polyacrylamide Gel

1. Prepare the stock electrophoresis 5x TBE buffer (Section 5). Dilute the stock to 1x.
2. Mix 10–15 µl of the sample with 5 µl of 5x dye buffer, heat to 65 °C for 5 min and load on a gel.
3. A 5% PAGE gel can separate DNAs from about 250 to 1,000 bp.
4. Run the gel in 1x TBE buffer at 100 V for 1–2 hours.

Standard Blot to Zeta-Probe

1. From the 5x TBE electrophoretic buffer, dilute the stock to 0.5x (Section 5) and pre-chill 1 L of the buffer.
2. Equilibrate the gel, extra thick blot paper, and Zeta-Probe membrane in 0.5x TBE buffer for at least 15 minutes.

   Note: Zeta-Probe membrane will bind non-denatured nucleic acids. Therefore, denaturing is not mandatory before transferring. If non-denatured nucleic acids are transferred, the blotted Zeta-Probe membrane must be treated with NaOH prior to hybridization. Refer to the Zeta-Probe membrane instruction manual.
3. Assemble the sandwich as described in Section 4.2.
4. Run the transfer at 400 mA for 1 hour (voltage should not exceed 25 volts).
5. After transfer, separate the membrane from the gel, and rinse the membrane briefly in 0.5x TBE buffer.
6. Fix the DNA to the membrane by placing the membrane on several pieces of blot paper saturated with 0.4 N NaOH for 10 minutes.
7. Rinse the membrane in 2 x SSC for 10 minutes and bake at 80 °C for 1 hour (this is optional if probing immediately). The membrane is now ready for hybridization. Refer to the hybridization procedure in the Zeta-Probe blotting membrane instruction manual.

6.3 DNA & RNA Blotting (For agarose gels with DNA up to 23 kb, RNA up to 3.5 kb)

Refer to the Trans-Blot SD DNA blotting kit instruction manual for transfer protocol and conditions. DNA or RNA cannot be blotted from agarose gels without the use of the Trans-Blot SD DNA blotting kit.

Section 7 Properties of Protein Blotting Media

PVDF membrane is suitable for presenting transferred proteins for immuno detection (Immun-Blot PVDF) or analysis by Edman. It is resistant to tearing and chemicals. Immun-Blot PVDF is optimized for immunodevelopment with high protein binding capacity (160 µg/cm^2), but low nonspecific protein binding. This membrane material will resist tearing even when used in repeated stripping and reprobing applications. Sequi-blot PVDF has the highest protein binding capacity (170–200 µg/cm^2) and gives outstanding performance in protein sequencing applications.

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Nitrocellulose membranes have been used extensively for protein binding and detection.[7,19-22] They can easily be stained for total protein by a dye stain (Amido Black, Coomassie® blue, Ponceau S, Fast Green FCF, etc.[22]), or the more sensitive Colloidal Gold Total Protein Stain, and also allow either RIA, FIA, or EIA.[7] Nitrocellulose has a high binding capacity of 80–100 µg/cm^2. Nonspecific protein binding sites are easily and rapidly blocked, avoiding subsequent background problems. Low molecular weight proteins (esp. < 20,000 daltons) may be lost during post transfer washes, thus limiting detection sensitivity.[21] However, use of glutaraldehyde fixation and a smaller pore size nitrocellulose membrane (0.2 µm) have been shown to be effective in eliminating this loss.[22] Large proteins (>100,000 daltons) denatured by SDS may transfer poorly with the addition of alcohol to the transfer buffer. Alcohol increases binding of SDS-proteins to nitrocellulose, but decreases pore sizes in the gel. Elimination of alcohol from SDS-protein transfers also results in considerably diminished binding to nitrocellulose. Under high field strengths of the Trans-Blot cell, proteins may be transferred through nitrocellulose without binding.The efficiency of binding can be increased by employing a smaller pore size nitrocellulose.[23]

Zeta-Probe positively charged nylon membrane allows binding of SDS-protein complexes in the absence of alcohol.[24,25] This membrane binds proteins very tightly and is stable to post transfer washes. The binding capacity of Zeta-Probe membrane is ~480 µg/cm^2. Reprobing, after stripping of prior probes, may be performed without significant loss of primary bound protein. Even small proteins appear to bind stably. Zeta-Probe membrane cannot be dye-stained, as destaining is impossible. Instead, the Biotin-Blot Total Protein Stain should be used on Zeta-Probe membrane. This assay uses NHS-Biotin (N-hydroxysuccinimide-biotinate) to biotinylate all the proteins on the membrane surface, and a combination of an avidin-horseradish peroxidase or avidin-alkaline phosphatase and a color development reagent to detect these biotinylated proteins.[26,27] The large capacity for molecules (480 µg/cm^2) allows sensitive detection of small amounts of proteins in a complex mixture. This high capacity requires more stringent blocking conditions than nitrocellulose.[25] Zeta-Probe membranes can be effectively and economically blocked using a 5% solution of BLOTTO (non-fat dry milk)[3,18,28]

Section 8
Troubleshooting Guide

8.1 Poor Transfer

A. Molecules remain in the gel matrix (as detected by Coomassie blue or silver staining the gel)

1. Transfer time is too short. Increase time of transfer.
2. Charge to mass ratio is incorrect. Proteins near their isoelectric point at the pH of the buffer will transfer poorly. Try a more basic or acidic transfer buffer to increase protein mobility.
3. Filter paper is too dry; insufficient buffer soaking the filter paper. Buffer is depleted early in the transfer. The filter paper should be fully saturated with buffer prior to transfer. Increase the number of sheets of filter paper, or use thicker filter paper.
4. Power supply circuit tripped. Check the fuse.
5. Gel percentage is too high. Reduce %T (total monomer) or %C (crosslinker). A 5% C (with bis as the crosslinker) will produce the smallest pore size gel. Decreasing from this concentration will increase pore size and increase transfer efficiency.

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6. Methanol in the transfer buffer is restricting elution of proteins from the gel. Elimination of methanol results in increased transfer efficiency, but it also diminishes binding to nitrocellulose. Use PVDF.

7. Protein is precipitating in the gel. Try using SDS in the transfer buffer. SDS can increase transfer efficiency, but can also reduce binding efficiency to nitrocellulose and affect reactivity of some proteins with antibodies.

B. Swirls or missing patterns on blot; diffuse transfers

1. Contact between blot membrane and gel is poor. Air bubbles or excess moisture remain between the blot and gel. Use a test tube or pipet to roll over the membrane carefully in both directions until excess moisture and air bubbles are removed from between gel and membrane and complete contact is established. Use thicker filter paper in the gel/membrane sandwich. Make sure that there are no air bubbles trapped between the filter paper and the gel.

2. The gel is not completely equilibrated in transfer buffer. Gel must be properly washed in transfer buffer to avoid shrinking or swelling during transfer. Increase time or number of washes.

3. If multiple gels are being transferred simultaneously, cross-contamination may be occurring. Use a smaller size pore dialysis membrane to separate gel/membrane sandwiches. Use PVDF to more completely bind small pieces.

4. Power conditions are too high. Reduce the voltage. Check the buffer conductivity; improperly prepared buffer will result in excessive power delivered to the cell.

8.2 Poor Binding to Nitrocellulose Membrane

1. Proteins separated by SDS-PAGE require 20% methanol in the transfer buffer for optimal protein binding. Make sure the buffer contains the proper amount of methanol.

2. Proteins may be transferring through the nitrocellulose, driven by the high field strength of the plate electrodes. Use Zeta-Probe membrane (higher binding capacity) or 0.2 micron nitrocellulose (smaller pore size). Transfer using the Trans-Blot cell or the Mini Trans-Blot cell with standard platinum wire electrodes.

3. Protein >15,000 daltons may show diminished binding to 0.45 micron nitrocellulose, or may be washed from the membrane during assays. Use Zeta-Probe membrane or 0.2 micron nitrocellulose. To increase stability of binding, proteins can be cross-linked to nitrocellulose with glutaraldehyde.[22]

4. Proteins can be removed from nitrocellulose by SDS, NP-40, and several other detergents. Use Tween-20 detergent in wash and antibody incubation steps. Reduce or eliminate detergents from buffers. Try glutaraldehyde fixation.

5. SDS in the transfer buffer will reduce binding efficiency of proteins. Use 20% methanol in the transfer buffer and equilibrate the gel in methanol buffer prior to transfer.

8.3 High Background After Incubation with Antibody Probes; Nonspecific or Nonquantitative Detection

For a complete troubleshooting guide to Immun-Blot assays, consult the Immun-Blot assay kit manual or the Zeta-Probe instruction manual. If using other detection kit, consult manual or contact manufacturer.

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8.4 Poor Detection Sensitivity or No Reactivity

1. Consult detection kit manual.
2. Antigen binding is incomplete. See Troubleshooting Sections 8.1–8.3.
3. Antibody reaction times are insufficient. Increase reaction times.
4. Sample load is insufficient. Increase the protein concentration applied to the gel.
5. Antigen may require specific temperature regulation during transfer to prevent denaturation. Use the Trans-Blot cell with the super cooling coil to transfer heat-sensitive proteins.
6. Monoclonal antibodies might not recognize a denatured antigen. Assess binding of other monoclonals or polyclonal antibodies. Blot native proteins.

Section 9
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