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# **Mini Trans-Blot<sup>®</sup> Electrophoretic Transfer Cell**

## **Instruction Manual**

Catalog numbers

170-3930

170-3935

170-3989

170-3836

**BIO-RAD**



## **Assembly and Disassembly**

To insure best performance from the Mini Trans-Blot® 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. After these preliminary steps, you should be ready to transfer a sample.

## **Wash Cell Before Use**

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

## **Warranty**

Bio-Rad Laboratories warrants the Mini Trans-Blot 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.

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 in 1975 with the transfer of DNA from agarose gels to nitrocellulose membranes.<sup>1</sup> Since that time, blotting has been applied to RNA<sup>2-4</sup> and proteins<sup>5, 6</sup> in both agarose and polyacrylamide gels. To circumvent the inefficiencies observed in various capillary transfers, electric current has been adopted for eluting proteins from polyacrylamide gels, as first described by Towbin *et al.* in 1979.<sup>7</sup> The use of electrophoretic transfer has also been applied to DNA and RNA blotting.<sup>8-14</sup> Numerous publications have dealt with the topic of protein electrophoretic transfer techniques.<sup>15-26</sup> There have also been reviews summarizing the expanding literature being generated on electrophoretic blotting methodology.<sup>27-29</sup>

The Mini Trans-Blot® tank is part of Bio-Rad's modular Mini-PROTEAN® Tetra system. The unique feature of this electrophoresis system is that the electrode modules are interchangeable. After finishing gel electrophoresis, remove the electrode module from the buffer tank, insert a new electrode module, add new buffer, and the next electrophoresis application can be performed.

The Mini Trans-Blot module accommodates two cassettes for electrophoretic transfer. The Mini Trans-Blot module is useful for blotting either protein or nucleic acid from both agarose and acrylamide gels. It is also capable of blotting isoelectric focusing gels from horizontal electrophoresis cells, or DNA and RNA gels from the Mini-Sub® submarine electrophoresis cell. For applications where the gel is larger than 7.5 x 10 cm, or when there are more than two mini gels to be transferred, the larger standard Trans-Blot® cell (catalog #170-3910 or 170-3946), Criterion™ Blotter (catalog #170-4070, 170-4071) or the Trans-Blot® SD semi-dry cell (catalog #170-3940) should be used.

The heart of the Mini Trans-Blot cell is its electrode module. This module has the capacity to hold two gel cassettes between parallel electrodes only 4 cm apart. The driving force for blotting applications is the voltage applied over the distance between the electrodes.

This short 4 cm electrode distance allows generation of higher driving forces to produce efficient protein transfers. A second feature of the electrode module is that it is offset to accommodate a blue cooling unit. The cooling unit, which is completely contained within the Mini Trans-Blot cell, absorbs the Joule heat generated during rapid electrophoretic transfers. The advantages of having an internal cooling unit include elimination of an expensive external cooling bath and avoidance of cumbersome cooling tubing. Other features of the Mini Trans-Blot cell include gel holder cassette latches for easy handling, color coordinated cassettes and electrodes to insure proper orientation of the gel during transfer, and an efficient design which simplifies insertion and removal of the cassettes from the electrode assembly. These features result in an electrophoretic transfer system which is easy to use and produces excellent blotting results.



## 1.1 Specifications

### Construction

Electrode module	Molded polysulfone
Gel holder cassettes	Molded polycarbonate
Electrodes	Platinum wire 0.254 mm diameter
Buffer chamber and lid	Molded polycarbonate
Cooling unit	Polyethylene

### Overall dimensions

Mini Trans-Blot cell	16 (L) x 12 (W) x 18 (H) cm
Gel holder dimensions	10 x 11 cm

### Maximum gel size

7.5 x 10 cm

### Buffer capacity

With cooling unit	950 ml
Without cooling unit	1,150 ml

### Cleaning

Use mild soap and warm water to clean the electrodes, cassettes, and buffer tank. Use special care when cleaning the electrode cards. Avoid stretching or breaking the platinum wires. Do not use abrasives or strong detergents. Rinse the fiber pads under hot water and then in distilled, deionized water.

### Chemical compatibility

The Mini Trans-Blot cell 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.**

## 1.2 Safety Instructions



Power to the Mini Trans-Blot cell is supplied by an external DC voltage power supply. This power supply must be ground isolated in such a way that the DC voltage output floats with respect to ground. All of Bio-Rad's power supplies meet this important safety requirement. Regardless of which power supply is used, the maximum specified operating parameters for the cell are:

**400 VDC**      Maximum voltage limit

**500 W**        Maximum power limit

**40°C**         Maximum ambient temperature limit



Current to the cell, provided from the external power supply, enters the unit through the lid assembly, providing a safety interlock to the user. Current to the cell is broken when the lid is removed. Do not attempt to circumvent this safety interlock, and always turn the power supply off before removing the lid, or when working with the cell in any way.

**Important:** This Bio-Rad instrument is designed and certified to meet IEC61010-1 and EN61010-1\* 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 IEC61010-1 and EN61010-1 safety certification**
- **Create a potential safety hazard**

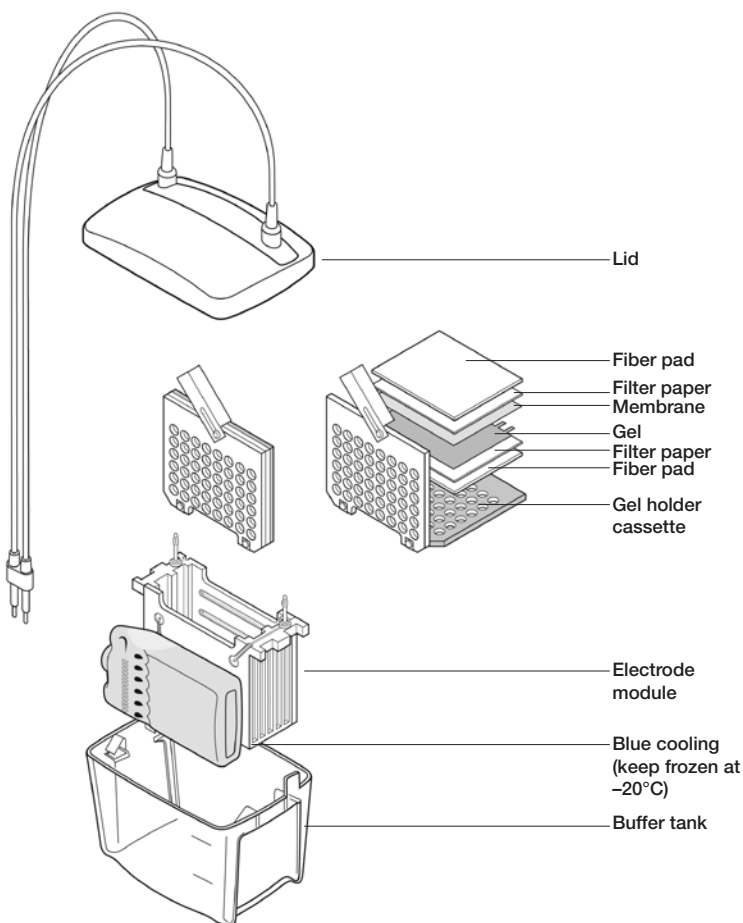
Bio-Rad is not responsible for any injury or damage caused by the use of this instrument for purposes other than for which it is intended or by modifications of the instrument not performed by Bio-Rad or an authorized agent.

\* IEC61010-1 and EN61010-1 are internationally accepted electrical safety standard for laboratory instruments.

## Section 2

### Mini Trans-Blot® Cell Assembly and Preparation for Transfer

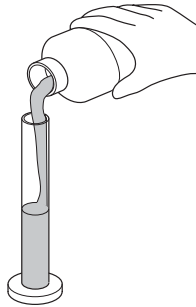
#### 2.1 Mini Trans-Blot Cell Description and Assembly of Parts



## 2.2 Preparation for Blotting

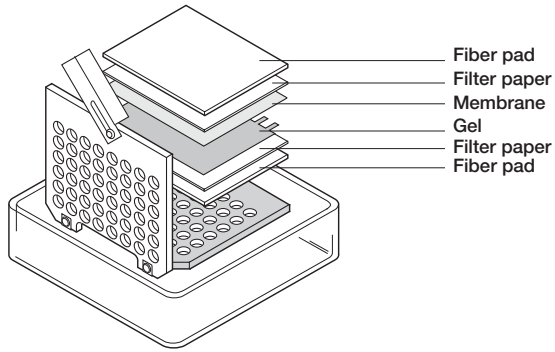
Store the blue cooling unit in your laboratory freezer at  $-20^{\circ}\text{C}$  until ready to use. After use, rinse the outside container with water and return the cooling unit to the freezer for storage.

1. Prepare the transfer buffer. (See Section 3.3 for buffer formulation. Using buffer chilled to  $4^{\circ}\text{C}$  will improve heat dissipation.)

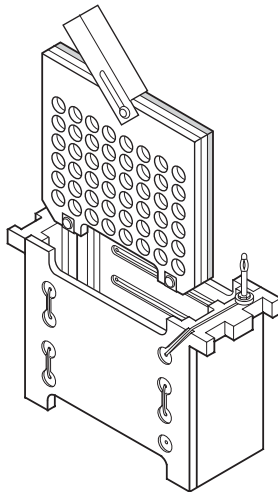


2. Cut the membrane and the filter paper to the dimensions of the gel or use precut membranes and filter paper. Always wear gloves when handling membranes to prevent contamination. Equilibrate the gel and soak the membrane, filter paper, and fiber pads in transfer buffer (15–20 min depending on gel thickness).
3. Prepare the gel sandwich.
  - a. Place the cassette, with the gray side down, on a clean surface.
  - b. Place one prewetted fiber pad on the gray side of the cassette.
  - c. Place a sheet of filter paper on the fiber pad.
  - d. Place the equilibrated gel on the filter paper.\*
  - e. Place the prewetted membrane on the gel.\*
  - f. Complete the sandwich by placing a piece of filter paper on the membrane.\*
  - g. Add the last fiber pad.

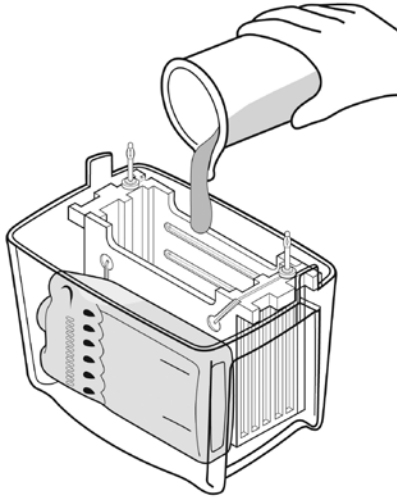
\* Removing any air bubbles which may have formed is very important for good results. Use a glass tube or roller to gently roll out air bubbles.



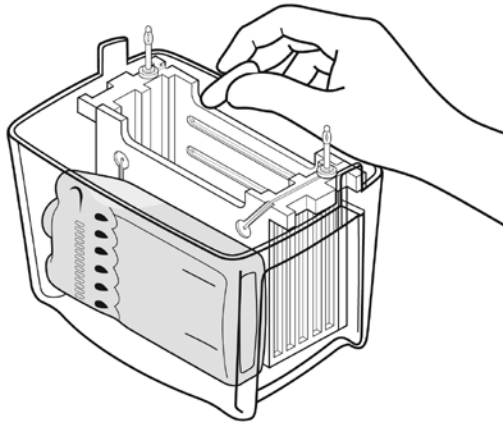
4. Close the cassette firmly, being careful not to move the gel and filter paper sandwich. Lock the cassette closed with the white latch.
5. Place the cassette in module. Repeat for the other cassette.



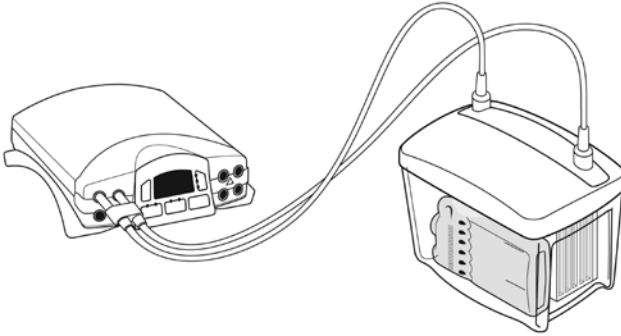
6. Add the frozen blue cooling unit. Place in tank and fill to the “blotting” mark on the tank.



7. Add a standard stir bar to help maintain even buffer temperature and ion distribution in the tank. Set the speed as fast as possible to keep ion distribution even.



- Put on the lid, plug the cables into the power supply, and run the blot. Refer to Section 3 for run times and voltage settings with various buffers.



- Upon completion of the run, disassemble the blotting sandwich and remove the membrane for development. Clean the cell, fiber pads, and cassettes with laboratory detergent and rinse well with deionized water.

### 2.3 Acidic Transfers

If transferring under acidic conditions, switch the gel and membrane in the set up instructions. This will place the membrane on the cathode side of the gel. Under acidic conditions, proteins will transfer in the opposite direction going toward the negative cathode.

## Section 3

### Transfer Conditions

#### 3.1 General Guide to Transfer Buffers and Running Conditions

Table 3.1 provides guidelines for power conditions using different buffers. Power conditions are provided for various run times. Where multiple conditions are displayed, the higher the voltage, the less time required for the run. Always use the blue cooling unit.

**Table 3.1. Guide to Buffers and Running Conditions.**

Buffer	Standard Field High Intensity Field Buffer Overnight Transfer	High Intensity Field 1 Hour Transfer
<b>SDS-PAGE Gels</b>		
<b>A:</b> 25 mM Tris, pH 8.3, 192 mM glycine, with or without 20% MeOH and .025%–0.1% SDS	30 V, constant 90 mA	100 V, constant 350 mA
<b>B:</b> 48 mM Tris, pH 9.2, 39 mM glycine, with or without 20% MeOH and .025%–0.1% SDS		
<b>C:</b> 10 mM NaHCO <sub>3</sub> , 3 mM NaCO <sub>3</sub> , pH 9.9, with or without 20% MeOH and .025%–0.1% SDS		
<b>DNA and RNA</b>		
<b>TAE:</b> 20 mM Tris, pH 7.8, 10 mM	30 V, constant 100 mA	80 V, constant 500 mA
<b>TBE:</b> 50 mM Tris, pH 8.3, 50 mM sodium borate, 1.0 mM EDTA		
<b>Native Gels</b>		
25 mM Tris, pH 8.3, 92 mM glycine. No methanol.	30 V, constant 90 mA	100 V, constant 350 mA
<b>Isoelectric Focusing, Native Gels, Basic Proteins, Acid Urea Gels*</b>		
0.7% acetic acid	30 V, constant 100 mA	100 V, constant 350 mA

\*Please refer to Section 2.3 before transferring.



### 3.2 Notes on Electrophoretic Transfer Conditions

**These variables will change total resistance and thus the current readings:**

- Alterations in buffer make-up, i.e., addition of SDS, or changes in ion concentration due to addition of acid or base to adjust the pH of the buffers
- Gel pH, ionic strength, and percentage of acrylamide, especially if the gel has not been properly equilibrated
- Number of gels; current increases slightly as the number of gels increases
- Volume of buffer; current increases when volume increases
- Platinum mass; current increases when mass increases
- Transfer temperature; current increases when temperature increases
- Time in transfer at which reading was taken; current normally increases as the buffering capacity diminishes with progress of the run

#### **Pre-equilibration of gels (15–20 min)**

All electrophoresis gels should be pre-equilibrated in transfer buffer prior to electrophoretic transfer.

Pre-equilibration will facilitate the removal of contaminating electrophoresis buffer salts and neutralization salts (salts resulting from the denaturation of nucleic acids prior to transfer). 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 will shrink in methanol buffers. Equilibration allows the gel to adjust to its final size prior to electrophoretic transfer.

#### **Current limits**

The PowerPac™ Basic power supply is capable of a 75 W output. Unless a current limit is set, uncontrolled conductivity changes may result in full power being delivered to the Mini Trans-Blot® cell.

The gel holders may warp, and the transfer buffer may boil and evaporate (further increasing conductivity). This would result in a potential safety hazard. Refer to the PowerPac Basic power supply instruction manual for setting current limits and run times. The Mini Trans-Blot cell is also compatible with the PowerPac HC power supply.

### **Use of a stir bar during transfer**

For all blotting applications a stir bar must be placed inside the Mini Trans-Blot cell and the entire unit be placed on a stir bar mixer, so that the transfer buffer is stirred during the course of the experiment. This will help to maintain uniform conductivity and temperature during electrophoretic transfer. Failure to properly control transfer buffer temperature results in poor transfer of macromolecules and poses a potential safety hazard.

### **Transfer buffer pH**

Do not adjust the pH of transfer buffers unless specifically indicated. Adjustments of the transfer buffers pH, when not indicated, will result in increased buffer conductivity. This is manifested by a higher than expected initial current output and a decreased resistance. It is recommended that the buffer conductivity and resistance be checked with the PowerPac Basic power supply before starting each transfer.

### **Transfer buffer recommendations**

Use only high quality, reagent grade methanol. Contaminated methanol can result in increased transfer buffer conductivity, as well as poor transfer of macromolecules. Do not reuse transfer buffers or dilute transfer buffers below recommended levels. Reuse of transfer buffers is not advised, since these buffers have most likely lost their ability to maintain a stable solution pH during transfer. Dilution of transfer buffers below their recommended levels is also not advised, since this will decrease buffering capacity.

## Voltage limits

Do not increase voltage settings beyond those indicated in Table 3.1. If overnight transfers at low voltages are ineffective for your application, and higher voltages are necessary, transfer times must also be decreased. Failure to do so may result in a potential safety hazard.

## 3.3 Buffer Formulation

All formulas provided below are for a total volume of 1 L of buffer. Approximately 950 ml of buffer are required for the Mini Trans-Blot cell with cooling unit. Ethanol can be used in place of methanol in all buffer formulations.

**Do not add acid or base to adjust pH of the following buffers.** Methanol should be analytical reagent grade, as metallic contaminants in low grade methanol will plate on the electrodes.

**Note:** Some pH electrodes will not perform a proper measurement for the pH of Tris buffers. If the pH of the buffer is off, check to make sure the electrode is designed to work with Tris buffers. If the pH electrode functions properly for Tris buffers and the pH is below 8.0, remake the buffer.

### **25 mM Tris, 192 mM glycine, 20% v/v methanol, pH 8.3**

Mix 3.03 g Tris, 14.4 g glycine, and 200 ml of methanol; add distilled deionized water (ddH<sub>2</sub>O) to 1 L.

### **25 mM Tris, 192 mM glycine, pH 8.3**

Mix 3.03 g Tris and 14.4 g glycine; add ddH<sub>2</sub>O to 1 L.

### **48 mM Tris, 39 mM glycine, 20% v/v methanol, pH 9.2**

Mix 5.82 g Tris and 2.93 g glycine in ddH<sub>2</sub>O, add 200 ml methanol.

Add to 1 L with ddH<sub>2</sub>O.

### **48 mM Tris, 39 mM glycine, pH 9.2**

Mix 5.82 g Tris and 2.93 g glycine.

Add ddH<sub>2</sub>O to 1 L.

**10 mM NaHCO<sub>3</sub>, 3 mM NaCO<sub>3</sub>, 20% methanol, pH 9.9**

Mix 0.84 g NaHCO<sub>3</sub> and 0.318 g NaCO<sub>3</sub> in ddH<sub>2</sub>O, add 200 ml methanol.

Add to 1 L with ddH<sub>2</sub>O.

**1.0x TBE (Tris-Borate EDTA), pH 8.3**

90 mM Tris-Borate, 1 mM EDTA

5x stock solution

54 g Tris base

27.5 boric acid

20 ml 0.5 M EDTA (pH 8.0)

Add 200 ml 5x stock solution to 800 ml ddH<sub>2</sub>O to make 1x working solution.

**1x TAE (Tris-Acetate EDTA)**

40 mM Tris-Acetate, 1 mM EDTA

50x stock solution

242 g Tris base

57.1 ml glacial acetic acid

100 ml 0.5 M EDTA (pH 8.0)

Add 20 ml 50x stock solution to 980 ml ddH<sub>2</sub>O to make 1x working solution.

## Section 4

# Strategies for Optimizing Electrophoretic Transfer

### 4.1 Optimizing Protein Transfer

Generally, quantitative elution of denatured high molecular weight proteins is difficult. The following tactics, alone or in combination, will increase transfer efficiency.

#### Vary gel composition

Gradient gels are often more effective than single gel concentrations for elution of a wide range of molecular weight proteins.

Lower the total monomer to create a more porous gel.

Increase or decrease the percentage of crosslinker. A 5.26% C gel will contain the smallest pore size of all gels no matter what the concentration of acrylamide. Decrease in %C will make gels more porous with little loss in resolution.

$$\%C = \frac{\text{grams bis}}{\text{grams bis} + \text{grams acrylamide}} \times 100$$

#### Increase transfer time

An initial control should be performed to determine the time required for complete transfer.<sup>18, 25</sup> Times may vary from as little as 30 minutes to as long as overnight. Remember all overnight applications should be performed at 30 volts to minimize heating problems.

#### Increase the power

Initial controls should be performed to evaluate the efficiency of increasing the V/cm as well as its effects on the temperature of transfer. The temperature increase may change buffer resistance and subsequent power delivered, as well as the state of protein denaturation, thus affecting transfer efficiency.

### **Reduce buffer strength**

Dilution of transfer buffer results in lower current at any given voltage. This will allow the use of higher voltages without excessive heating. However, be aware not to dilute the buffer below its buffering capacity.

### **Vary buffer type and pH**

Maximize charge-to-mass ratio. It appears that alcohols present in SDS transfer buffer strip SDS from proteins. Basic proteins in Tris, glycine, methanol buffer at pH 8.3 may assume a state near isoelectric neutrality and thus transfer poorly. For example, lysozyme exhibits this behavior. Buffers with pH of 9.5–10.0 have shown much better elution and binding characteristics for basic proteins such as lysozyme and histones.<sup>41</sup>

Different buffer types at similar V/cm may yield different efficiencies. Generally, Tris buffers allow more efficient transfer than acetate or phosphate buffers.

### **Add detergent**

Addition of 0.1% SDS detergent to Tris, glycine, methanol buffer has been reported to increase transfer efficiency.<sup>25</sup> SDS, however, increases relative current, power, and heating. Also, temperatures below 10°C may precipitate the SDS so the starting buffer temperature will be higher. SDS may also affect the antigenicity of some proteins. SDS will aid in eluting the proteins from the gel, but it may reduce the binding efficiency of those proteins to the membrane.

### **Eliminate alcohol from the transfer buffer**

Alcohol in the transfer buffer improves binding of proteins to nitrocellulose only. Elimination of alcohol results in increased transfer efficiency but diminishes binding to nitrocellulose. Transfer efficiency is increased because alcohol causes gel pores to contract resulting in capture of large molecular weight proteins within the gel matrix.

Use of PVDF membrane for protein transfers eliminates the alcohol requirement, and constitutes a logical strategy for analysis of high molecular weight or difficult-to-transfer proteins.<sup>27, 28</sup> PVDF must be wetted in 100% methanol but may then be used in buffer without methanol.

### **Limited protease treatment**

A protocol for protease digestion of protein during transfer has been published.<sup>23</sup> Efficient transfer without loss of immunological reactivity was reported.

### **Alter membrane type**

Both nitrocellulose and PVDF can be used for protein transfer.

### **Alter gel system**

If possible, use nondenaturing gradient pore gels for separation of proteins. Isoelectric focusing gels, or native gels, may be considered if separation by molecular weight is not mandatory.

### **Enhance gel-membrane contact**

Failure of molecules to bind efficiently to the membrane, caused by poor gel-membrane contact, is often confused with inefficient elution. Poor contact is usually due to excess moisture in the gel-membrane interface. Proper technique and the use of a test tube or glass pipet as a “rolling pin” should assure good contact. Proper selection of filter paper spacers will help assure good compression. Gel and membrane equilibration in transfer buffer for 15–20 min prior to transfer will help prevent shrinking of either component during transfer, and will eliminate reactants such as urea or SDS from the gel.

## **4.2 Optimizing DNA and RNA Transfer**

Problems with elution of nucleic acids can be solved by altering the gel percentage. It may be somewhat more difficult to quantitatively transfer large amounts of DNA used in genomic blots. Agarose gels over 6 mm thick are not compatible with the Mini Trans-Blot. The following tactics should be considered for optimizing elution in such transfers.

### **Alter gel composition**

Lower % total monomer or % crosslinker for polyacrylamide gels.

Lower % agarose. This allows better elution of high molecular weight DNA.

### **Alter DNA denaturants**

It has been found that glyoxal denaturation allows more efficient elution of DNA than NaOH. Boiling polyacrylamide gels to denature DNA has also been found to give excellent results.<sup>12</sup> Base denaturation often causes polyacrylamide gels to weaken and stick to blotting membranes.



## Section 5

### Choice of Blotting Membranes

#### 5.1 Protein Blotting Membranes

##### Nitrocellulose Membrane

Nitrocellulose membranes have been used extensively for protein binding and detection.<sup>8, 21, 24, 25, 28</sup> They can be easily stained for total protein by a dye stain (Amido Black, Coomassie Blue, Ponceau S, Fast Green FCF, etc.),<sup>28</sup> or the more sensitive Colloidal Gold Total Protein Stain, and also allow either RIA, FIA, or EIA.<sup>8</sup> Nitrocellulose has a high binding capacity of 80–100 µg/cm<sup>2</sup>. Nonspecific protein binding sites are easily and rapidly blocked, avoiding subsequent background problems. No pre-activation is required. Low molecular weight proteins (especially <15,000 daltons) may be lost during post transfer washes, thus limiting detection sensitivity.<sup>20</sup> Smaller pore size nitrocellulose membrane (0.2 µm), has been shown to be effective in eliminating this loss.<sup>30</sup> Large proteins (>100,000 daltons) denatured by SDS may transfer poorly due to 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 results in considerably diminished binding. Adding SDS (up to 0.1%) to the transfer buffer increases the transfer efficiency of proteins, but reduces the amount of binding to the membrane.<sup>18</sup> Also, SDS increases the conductivity of the buffer and the heat generated during transfer.

##### PVDF Membrane

Polyvinylidene difluoride (PVDF) membrane is an ideal support for amino-terminal sequencing, amino acid analysis and immunoassays of blotted proteins. PVDF retains proteins under extreme conditions of exposure to acidic or basic conditions, and in the presence of organic solvents.

Greater retention during sequencing manipulations enhances the likelihood of obtaining information from rare, low abundance proteins, by increased initial coupling and higher repetitive yields. In addition, PVDF membrane exhibits better binding efficiency of blotted material in the presence of SDS in the transfer buffer. PVDF must first be wetted in 100% MeOH but can then be used in buffer, which does not contain MeOH.

## 5.2 DNA and RNA Blotting Membranes

### Zeta-Probe® Nylon Membrane

Nitrocellulose is not a suitable medium for electrophoretic transfer of nucleic acids, as high concentrations of salt (>10x SSC) are required for efficient binding.<sup>13</sup> Molecules ≤500 bp are not bound at all, even at high salt. Low resistance results when an electric current is passed through a solution of high salt. This causes potentially damaging high currents (and power) even at very low voltages. Since V/cm is the eluting force, inefficient transfer occurs under conditions required for proper binding. Zeta-Probe membrane allows efficient binding of all sizes of single stranded DNA and RNA in the presence of low ionic strength buffers.<sup>13</sup> Zeta-Probe membrane is an ideal alternative to nitrocellulose for the transfer of nucleic acids. Binding is more stable through post transfer washes, and reprobing may be performed as many as 10 times.

A variety of blotting membranes is available for immunoblotting, each with particular advantages depending on the needs of the experiment. The physical properties and performance characteristics of a membrane should be evaluated when selecting the appropriate transfer conditions.

**Table 5.1 Guide to Protein Blotting Membranes**

<b>Membrane</b>	<b>Pore Size</b>	<b>Binding Capacity (<math>\mu\text{g}/\text{cm}^2</math>)</b>	<b>Notes</b>
Nitrocellulose	0.45 $\mu\text{m}$ 0.2 $\mu\text{m}$	80–100	General purpose protein blotting membrane.
Supported Nitrocellulose	0.45 $\mu\text{m}$ 0.2 $\mu\text{m}$	80–100	Pure nitrocellulose cast on an inert synthetic support; increased strength for easier handling and for reprobing.
PVDF	0.2 $\mu\text{m}$	170–200	High mechanical strength and chemical stability, used for protein sequencing and western blotting; enhanced binding in the presence of SDS. Must be wet in alcohol before equilibration in buffer.
Nylon	0.2 $\mu\text{m}$	170	Recommended for nucleic acids.

**Note:** Nucleic acids cannot be transferred to nitrocellulose by electrophoretic blotting. Use Zeta-Probe membrane.

## Section 6

### Troubleshooting Guide

#### 6.1 Electrophoretic Transfer

##### Poor electrophoretic transfer (as detected by staining the gel)—proteins

1. Transfer time is too short.
  - Increase the transfer time
2. Power is too low.
  - Always check the current at the beginning of the run. The current may be too low for a particular voltage setting. If the buffer is prepared improperly, the conductivity may be too low, and not enough power will be delivered to the cell. See the power guidelines for specific applications in Section 3
  - Remake the buffer or increase the voltage
  - Try the high intensity blotting option
3. Power supply circuit is inoperative, or an inappropriate power supply was used.
  - Check the fuse. Be sure the voltage and current output of the power supply match the needs of the blotting instrument
4. Transfer apparatus is assembled incorrectly, and the proteins are moving in the wrong direction.
  - The gel/membrane sandwich may be assembled in the wrong order or the cassette is inserted in the tank facing the opposite orientation. Check the polarity of the connections to the power supply
  - Use a pre-stained protein standard to assess transfer efficiency after blotting

5. Charge-to-mass ratio is incorrect.
  - Try a more basic or acidic transfer buffer to increase protein mobility.
6. 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
  - An excess of methanol will lead to protein precipitation. Try decreasing methanol content
7. Methanol in the transfer buffer is restricting elution.
  - Reduction of methanol results in increased transfer efficiency of proteins from the gel, but it also diminishes binding to nitrocellulose
8. Gel percentage too high.
  - Reduce %T (total monomer) or %C (crosslinker). A 5.26% C (with bis as the crosslinker) will produce the smallest pore size gel. Decreasing from this concentration will increase the pore size and increase transfer efficiency

### **Poor transfer—nucleic acid**

1. Gel percentage is too high.
  - Reduce the %T or %C in the acrylamide gel or reduce % agarose in an agarose gel
  - Prior to transfer, cleave DNA in 0.25 M HCl or RNA in dilute NaOH
2. Transfer time is too short or power conditions are too low.
  - Increase the transfer time, or try high intensity transfer
3. DNA or RNA cannot be transferred electrophoretically to nitrocellulose, since high salt concentrations are required for efficient binding.
  - Use Zeta-Probe membrane instead of nitrocellulose

## Swirls or missing bands; diffuse transfers

1. Poor contact between the membrane and the gel. Air bubbles or excess buffer remain between the blot and gel.
  - Use a test tube or pipet as a rolling pin, and roll over the membrane carefully in both directions until air bubbles and excess buffer are removed from between gel and membrane, and complete contact is established
  - Use thicker filter paper in the gel/membrane sandwich
  - Replace the fiber pads. Pads will compress with time, and will not hold the membrane to the gel
2. Power conditions are too high.
  - Always check the current at the beginning of the run. The current may be too high for a particular voltage setting. If the buffer is prepared improperly, the conductivity may be too high, resulting in excessive power delivered to the cell. See the power guidelines for specific applications in Section 3
3. The membrane is not properly wet or has dried out.
  - White spots on the nitrocellulose membrane indicate dry areas where protein will not bind. If wetting does not occur immediately by immersion of the sheet in transfer buffer, heat distilled water until just under the boiling point, and soak the membrane until completely wet. Equilibrate in transfer buffer until ready for use
  - Because of the hydrophobic nature of PVDF, the membrane must be prewet in methanol prior to equilibration in aqueous transfer buffer. Do not let membrane dry after wetting. Rewet in methanol if necessary

4. The gel electrophoresis may be at fault.
  - Artifacts of electrophoresis may be produced by poor polymerization, inappropriate running conditions, contaminated buffers, sample overload, etc

### **Gel cassette pattern transferred to blot**

1. Contaminated or thin fiber pads are used.
  - Replace the fiber pads, or thoroughly clean the contaminated pads
2. Excessive amounts of protein were loaded on the gel, or too much SDS was used in the transfer buffer. Proteins can pass through the membrane without binding, and recirculate through the tank blotting system.
  - Reduce the amount of protein on the gel, and SDS in the transfer buffer. Reduce transfer duration or add a second sheet of membrane to bind excess protein
3. The transfer buffer is contaminated.
  - Make fresh solutions. Transfer buffer solution cannot be reused

### **Poor binding to the membrane—nitrocellulose**

1. Nitrocellulose requires 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.
  - Use PVDF (higher binding capacities) or 0.2  $\mu\text{m}$  nitrocellulose (smaller pore size). Decrease the voltage if using the high intensity option
3. Mixed ester celluloses bind proteins poorly.
  - Use pure nitrocellulose

4. Proteins <15,000 daltons may show diminished binding to 0.45  $\mu\text{m}$  nitrocellulose, or may be washed from the membrane during assays.
  - To increase stability of binding, proteins can be crosslinked to nitrocellulose with glutaraldehyde
  - Use PVDF membrane, which has higher binding capacities
  - Use Tween-20 detergent in the wash and antibody incubation steps. Reduce or eliminate the more stringent washing conditions
5. SDS in the transfer buffer will reduce binding efficiency of proteins.
  - Reduce or eliminate the SDS from the transfer buffer
6. The membrane may not be completely wet.
  - White spots on the membrane indicate dry areas where protein will not bind. If wetting does not occur immediately by immersion of the sheet in transfer buffer, heat distilled water until just under the boiling point, and soak the membrane until completely wet. Equilibrate in transfer buffer until ready for use

### **Poor binding to the membrane—PVDF**

1. The membrane may not be completely wet.
  - Because of the hydrophobic nature of PVDF, the membrane must be prewet in alcohol prior to equilibration in aqueous transfer buffer. Follow the directions in the product insert
2. The membrane may have been allowed to dry during handling.
  - A completely wet membrane has a gray, translucent appearance. White spots will form on the surface of the membrane, indicating that it has been allowed to dry. Since proteins will not bind to the dry spots, rewet the membrane with methanol and re-equilibrate in transfer buffer



## Section 7

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## Section 7

### Product Information

Catalog Number	Product Description
<b>Mini Trans-Blot® Cell</b>	
170-3930	Mini Trans-Blot Electrophoretic Transfer Cell, includes 2 gel holder cassettes, 4 fiber pads, modular electrode assembly, blue cooling unit, lower buffer chamber, and lid with cables
170-3935	Mini Trans-Blot Module, same as 170-3930 without lower buffer chamber and lid
170-3989	Mini Trans-Blot Cell and PowerPac Basic Power Supply
170-3836	Mini Trans-Blot Cell and PowerPac HC Power Supply
<b>Mini Trans-Blot Cell Accessories</b>	
170-3931	Mini Gel Holder Cassette
170-3932	Filter Paper, 7.5 x 10.5 cm, 50
170-3933	Fiber Pads, 8 x 11 cm, 4
170-3934	Bio-Ice™ Cooling Unit

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