

Increased Transfer Efficiency using a Discontinuous Buffer System with the Trans-Blot® SD Semi-Dry Electrophoretic Transfer Cell

By Anthony K. Tan, Ph.D., Bio-Rad Laboratories, Hercules, CA, USA

Introduction

Semi-dry transfer has traditionally been a fast but inefficient method for transferring all of the protein from a gel onto a membrane. By using a discontinuous buffer system, transfer efficiency can be greatly increased. In protein blotting, the buffer has an important effect on the elution of proteins from a gel and on the retention of proteins on the membrane.

A unique feature of semi-dry blotting is the ability to use two different buffers during transfer, which is termed a discontinuous buffer system. This is important because methanol and SDS, commonly used in transfer buffers, have opposite effects on binding of proteins to gel and membrane (see discussion below). Optimization of the transfer buffer composition can be accomplished using a discontinuous buffer system.

The Effects of Methanol

Methanol increases a protein's affinity for a membrane by removing bound SDS from the protein. This increases the number of hydrophobic sites on the protein that are available to bind to the membrane support. It is for this reason that methanol is often used in transfer buffers. While advantageous for binding of proteins to the membrane, methanol causes the pores in the gel to constrict. This makes it mechanically more difficult for proteins to exit the gel.

The Effects of SDS

SDS is used in western blotting transfer buffer to aid in the elution of proteins from the gel matrix. While it inhibits the binding of proteins to the membrane, it is often necessary to facilitate complete transfer of proteins from the gel.

Using a Discontinuous Buffer System

The opposing effects of methanol and SDS in blotting can be exploited in semi-dry transfer because the buffer reservoirs (the filter paper on both sides of the gel) are independent. In a discontinuous system, methanol should be included in the buffer on the membrane side (anode) of the blot assembly and SDS included on the gel side (cathode), taking advantage of the beneficial effects of each component.

A discontinuous buffer system using Tris/CAPS buffers in the Trans-Blot SD semi-dry electrophoretic transfer cell provides excellent results in semi-dry blotting. This procedure uses Tris/CAPS buffer plus 15% methanol in the filter paper at the anode and Tris/CAPS plus 0.1% SDS in the filter paper at the cathode.

The effectiveness of the technique is illustrated in Figure 1. Cytosolic protein from white blood cells was separated by SDS-PAGE (Figure 1A); following transfer to a membrane (1B), the p47phox component of the respiratory burst oxidase was detected by western blotting using Immun-Star™ chemiluminescent substrate (1C).

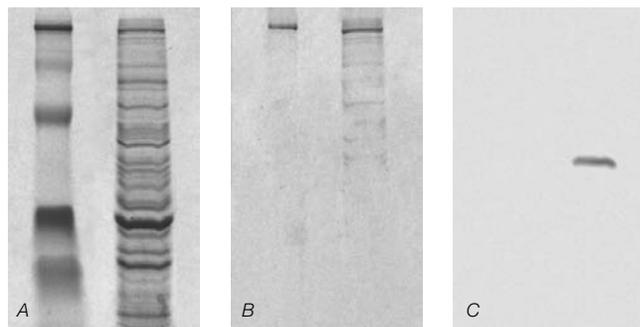


Fig. 1. Efficient transfer using the Tris/CAPS discontinuous buffer system. A, SDS-PAGE of white blood cell cytosol. Lane 1, Bio-Rad Kaleidoscope precast standards; lane 2, total cytosolic protein (18 µg) run on Bio-Rad precast 10% polyacrylamide gel stained with Coomassie® Blue R-250. B, Gel identical to gel in A, blotted onto PVDF membrane using the Trans-Blot semi-dry transfer cell, then stained with Coomassie Blue. C, Western blot of p47phox protein detected by Immun-Star chemiluminescence.

For Best Results

Bio-Rad's extra thick blotting paper should be used. This paper is 2.6 mm thick 100% cotton fiber, and provides the absorbency required for semi-dry blotting.

PVDF provides superior retention of protein. For proteins with molecular weight less than 20 kD, PVDF is required to prevent loss of proteins due to "blow-through" during electrophoretic transfer. Its higher binding capacity will increase detection sensitivity. It is important to first wet the PVDF in 100% methanol, then equilibrate in anode buffer for at least 30 min prior to transfer. It is expedient to begin equilibration during the vertical electrophoresis run.

TRIS/CAPS BUFFER FORMULATION

5x stock solution:

36.34 g Tris base
44.26 g CAPS
Water to 1 L

To prepare 100 ml of each working buffer from the 5x stock solution (final concentration 60 mM Tris, 40 mM CAPS, pH 9.6):

Anode (bottom) buffer:	Cathode (top) buffer:
20 ml 5x Tris/CAPS	20 ml 5x Tris/CAPS
15 ml MeOH	1 ml 10% SDS
65 ml water	79 ml water

TRANSFER CELL ASSEMBLY

Following vertical electrophoresis:

1. Wet the PVDF membrane in 100% MeOH, then equilibrate in anode buffer for at least 30 min. To this buffer add one sheet of extra thick blotting paper.
2. Equilibrate the polyacrylamide gel in cathode buffer. Soak a second piece of extra thick blotting paper in this buffer.
3. Prepare for transfer by assembling the gel sandwich in the following order:
 - Platinum (bottom) anode platform
 - Extra thick filter paper previously soaked in anode buffer
 - Equilibrated PVDF membrane
 - Equilibrated gel
 - Extra thick filter paper previously soaked in cathode buffer
4. Securely latch top stainless-steel cathode assembly, cover with safety lid, and run at constant current, 1.5 mA per square centimeter of gel (for example, 120 mA for a small 8 x 10 cm gel) for 30–60 min.

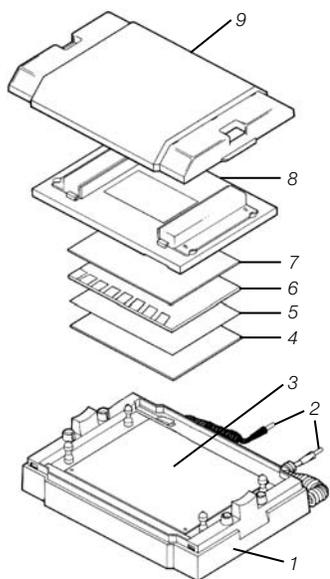


Fig. 2. Exploded view of the Trans-Blot SD semi-dry electrophoretic transfer cell. 1, Base; 2, power cables; 3, spring-loaded anode platform, mounted on four guideposts; 4, filter paper; 5, membrane; 6, gel; 7, filter paper; 8, cathode assembly with latches; 9, safety lid.

Bibliography

- Gallagher, S. R., et al., *Immunoblotting and immunodetection*, in *Current Protocols in Molecular Biology* (Ausubel, F. M., et al., eds.), pp. 10.8.1–10.8.16, Greene Publishing and Wiley Interscience, New York (1992)
- Hirano, H., *Microsequence analysis of winged bean seed proteins electroblotted from two-dimensional gel*, *J. Protein Chem.* **8**, 115–130 (1989)
- Jacobson G., *Protein blotting using semi-dry electrophoretic transfer equipment*, in *Protein Blotting* (Dunbar, B. S., ed.), pp. 53–70, IRL Press, New York (1994)
- Kyhse-Andersen, J., *Electroblotting of multiple gels: a simple apparatus without buffer tank for rapid transfer of proteins from polyacrylamide to nitrocellulose*, *J. Biochem. Biophys. Methods*, **10**, 203–209 (1984)
- Lauriere, M., *A semidry electroblotting system efficiently transfers both high- and low-molecular-weight proteins separated by SDS-PAGE*, *Anal. Biochem.*, **212**, 206–211 (1993)
- Lissilour, S. and Godinot, C., *Influence of SDS and methanol on protein electrotransfer to Immobilon P membranes in semidry blot systems*, *BioTechniques*, **9**, 397–401 (1990)
- Mozdzanowski, J. and Speicher, D. W., *Quantitative electrotransfer of proteins from polyacrylamide gels onto PVDF membranes*, in *Current Research in Protein Chemistry: Techniques, Structure, and Function* (Villafranca, J., ed.), pp. 87–93, Academic Press, San Diego (1990)
- Patterson, S. D., *From electrophoretically separated protein to identification: strategies for sequence and mass analysis*, *Anal. Biochem.*, **221**, 1–15 (1994)
- Smejkal, G. and Gallagher, S., *Determination of semidry protein transfer efficiency with transverse gradient gel electrophoresis*, *BioTechniques*, **16**, 196–202 (1994)
- Svoboda, M., et al., *Rapid electrotransfer of proteins from polyacrylamide gel to nitrocellulose membrane using surface-conductive glass as anode*, *Anal. Biochem.*, **151**, 16–23 (1985)
- Tovey, E. R. and Baldo, B. A., *Comparison of semi-dry and conventional tank-buffer electrotransfer of proteins from polyacrylamide gels to nitrocellulose membranes*, *Electrophoresis*, **8**, 384–387 (1987)
- Vaessen, R. T., et al., *Protein transfer to nitrocellulose filters. A simple method for quantitation of single proteins in complex mixtures*, *FEBS Lett.*, **124**, 193–196 (1981)

Ordering Information

Catalog #	Description
<i>Trans-Blot SD Cell and System</i>	
170-3940	Trans-Blot SD Semi-Dry Electrophoretic Transfer Cell
170-3948	Trans-Blot SD System, 100/120 V, includes PowerPac 200 power supply
170-3949	Trans-Blot SD System, 220/240 V
<i>Immun-Star Kits</i>	
170-5010	Goat Anti-Mouse Detection Kit
170-5011	Goat Anti-Rabbit Detection Kit
170-5012	Substrate Pack
170-5018	Substrate Only
170-5013	Goat Anti-Mouse Intro Kit
170-5014	Goat Anti-Rabbit Intro Kit
170-5015	Blotting Reagents Pack
<i>Blot Absorbent Filter Paper (Extra Thick)</i>	
170-3965	Extra Thick Blot Paper, 7.5 x 10 cm, 60
170-3958	Extra Thick Blot Paper, 10 x 15 cm, 30
170-3959	Extra Thick Blot Paper, 15 x 15 cm, 30
170-3960	Extra Thick Blot Paper, 15 x 20 cm, 30

BIO-RAD

**Bio-Rad
Laboratories**

Life Science
Group

Web Site www.bio-rad.com U.S. (800) 4BIORAD Australia 02 9914 2800 Austria (01)-877 89 01 Belgium 09-385 55 11 Canada (905) 712-2771
China 86-10-62051850/51 Denmark 45 39 17 99 47 Finland 358 (0)9 804 2200 France 01 43 90 46 90 Germany 089 318 84-0
Hong Kong 852-2789-3300 India (91-11) 461-0103 Israel 03 951 4127 Italy 39-02-216091 Japan 03-5811-6270 Korea 82-2-3473-4460
Latin America 305-894-5950 Mexico 514-2210 The Netherlands 0318-540666 New Zealand 64-9-4152280 Norway 22-74-18-70 Russia 7 095 979 98 00
Singapore 65-2729877 Spain 34-91-661-7085 Sweden 46 (0)8-55 51 27 00 Switzerland 01-809 55 55 United Kingdom 0800-181134