3.3 Acidic Transfers

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

1. If the standard electrode cards are used, assemble the gel sandwich as in Section 3.2 Reverse the polarity by placing the red cable into the black power supply outlet and the black cable into the red outlet. Turn on the power supply. Refer to Section 4.1 for transfer conditions.

2. **If the plate electrodes are used, do not reverse the polarity.** This will result in damage to the electrodes. Place the cassette into the tank so that the gray panel of the gel holder faces the anode (the red card). Plug the red cable into the red power supply outlet and the black cable into the black outlet. Turn on the power supply. Refer to Section 4.1 for transfer conditions.

3.4 High Intensity Field Option

The electrode panels of the Trans-Blot cell can be moved closer together to allow higher field strengths (V/cm), and thus more efficient or rapid transfer. To decrease the inter-electrode distance, the anode electrode panel is moved to the center gel holder position.

1. Remove the screws at the top corners of the anode (red) electrode panel.

2. Set up the gel holder with the gel, pads, and membrane following the standard Trans-Blot cell operation instructions outlined in Section 3.1 and 3.2. Insert the sandwich into the gel holder slot closest to the cathode (black) electrode panel.

3. Pull the anode panel out of the cell, and move it into the middle gel holder slot. The gel holder will keep the electrode panel firmly positioned in the slot.

4. Completely fill the buffer tank to the bottom of the red electrode disc.

5. Place the Super Cooling Coil into the free gel holder slot and place a stir bar in the bottom of the cell. **The Super Cooling Coil must be used for adequate heat dissipation of** [redacted]

6. Remove the white plug from the alternate electrode cable hole in the Trans-Blot cell lid. Unscrew the two red screws anchoring the red power cable to the lid. Loosen the screws 4 turns. Move the cable to it's alternate position, aligning shallow holds in the cable with the anchor screws, and retighten all screws.

7. Begin electrophoretic blotting.

Section 4
Transfer Conditions

4.1 General Guide for Transfer Buffers and Running Conditions

Table 4.1 gives the recommended buffers and voltage settings for electrophoretic transfers using the Trans-Blot cell. All transfers should be performed at constant voltage settings. A current limit should be set to avoid overheating the buffer. Do not substitute buffers (e.g., use the 25 mM Tris, 192 mM glycine, 20% v/v methanol buffer for transfers from SDS-PAGE gels to nitrocellulose; **do not substitute a phosphate buffer which is much more conductive and which will cause heating problems).** All starting voltage and current conditions presented in the table were determined for transfers using two gels and two cassettes, with [redacted] starting buffer. Transfer conditions should remain the same whether one or six gels are blotted. When a transfer is started, **the current observed should be similar to that described in the table for the**

given constant voltage setting. If the current is higher, then the buffer has been improperly made. The increased current from improperly made buffers will increase the Joule heat generated during the transfer, which will cause problems by overheating the buffer. Always monitor the current and buffer temperature closely for the first run of a new buffer system. Record the starting current for the specific voltage setting and use it as an indicator of proper buffer preparation for future applications.

1. The following variables will change total resistance and thus the current readings:
   a. Alterations in buffer make-up, i.e., addition of SDS, or changes in ion concentrations due to addition of acid or base to adjust the pH of the buffers.
   b. Gel pH, ionic strength, and percentage of acrylamide, especially if the gel has not been properly equilibrated.
   c. Number of gels; current increases slightly as the number of gels increases.
   d. Volume of buffer; current increases when mass increases.
   e. Platinum mass; current increases when mass increases.
   f. Transfer temperature; current increases when temperature increases.
   g. Time in transfer at which reading was taken; current normally increases as the buffering capacity diminishes with progress of the run.
2. If low field conditions are used overnight for convenience, it is advisable to transfer for 1-2 hours the following morning at standard or high intensity field conditions.
3. The buffer formulas are listed in Table 4.1 to avoid confusion, and are explained in more detail in Section 4.3. Some important points are made. For example, the pH of the Towbin buffer should not be adjusted. Tris acetate buffer becomes more conductive as the pH becomes more neutral and when pH adjustment is made with HCl.

4.2 Notes on Electrophoretic Transfer Conditions

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

Read the entire instruction manual before beginning electrophoretic transfers.

Do not attempt high intensity transfers or overnight runs until you are completely familiar with your system.

1. Pre-equilibration of gels

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 (<12%) will shrink in methanol buffers. Equilibration allows the gel to adjust to its final size prior to electrophoretic transfer.

2. **Current limits**

The Model 200/2.0 Power Supply is capable of a 200 watt output. **Unless a current limit is set, uncontrolled conductivity changes may result in full power being delivered to the Trans-Blot cell. The gel holder and electrode cards may deform, and the transfer buffer may boil and evaporate (further increasing conductivity). This would result in a potential safety hazard.** Refer to the Model 200/2.0 Power Supply Instruction Manual for setting current limits and run times.

3. **Polarity of transfer**

Do not reverse polarity with the plate electrodes. 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 cleanser to remove the rust.

4. **Heat dissipation with the Super Cooling Coil - Use of the Super Cooling Coil with the Plate Electrodes**

For high power applications, such as high intensity transfers and transfers performed with the plate electrodes, **efficient heat removal is only obtained using the Super Cooling Coil connected to a refrigerated recirculating bath.** Placing the Trans-Blot cell in the cold room is an inadequate means of controlling transfer buffer temperature. The plastic tank of the Trans-Blot cell is an effective thermal insulator, thus limiting the efficient dissipation of heat. **Use of the Super Cooling Coil is necessary with all experiments using the plate electrodes and with high intensity transfers using the standard platinum wire electrodes.**

5. **Use of a stir bar during transfer**

For all blotting applications a stir bar must be placed inside the Trans-Blot cell, 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.**

6. **Transfer buffer pH**

Do not adjust the pH of transfer buffers unless specifically indicated. Adjustments of the pH of transfer buffers, 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 Model 200/2.0 Power Supply before starting each transfer.

7. **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 their buffering capacity.

8. **Voltage limits**

Do not increase voltage settings beyond those indicated in Table [illegible] for overnight operation. Buffer conductivity must be close to the current listed in Table 4.1 and a current limit should be set on the power supply. 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.**

Table 4.1 General Guideline to Transfer Buffers and Running Conditions

Transfer	Buffer	Blot Media	Power Ranges: Standard Wire Electrode			Plate Electrode		
			[illegible]	[illegible]	[illegible]	[illegible]	Standard Field	High Intensity
			[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible] to 1 Hour
SDS-Protein (Laemmli, etc.) 2-D	25 mM Tris, pH 8.3, 192 mM glycine with 20% methanol:	Nitrocellulose	30 V, 0.1 A	60 V, 0.21 A	100 V, 0.36 A 150 V, 0.55 A 200 V, 0.85 A	15 V, 0.1 A	50 V, 0.5 A 100 V, 1.0 A 150 V, 1.5 A	50 V, 0.7 A 60 V, 0.9 A 100 V, 1.5 A
	Without methanol:	Zeta-Probe	30 V, 0.1 A	60 V, 0.21 A	100 V, 0.36 A 150 V, 0.55 A 200 V, 0.85 A	15 V, 0.1 A	50 V, 0.5 A 100 V, 1.0 A 150 V, 1.5 A	50 V, 0.7 A 60 V, 0.9 A 100 V, 1.5 A
	48 mM Tris, pH 9.2, 39 mM glycine with 20% methanol:	Nitrocellulose	30 V, 0.1 A	60 V, 0.2 A	100 V, 0.43 A 150 V, 0.67 A 200 V, 0.91 A	15 V, 0.1 A	50 V, 0.55 A 100 V, 1.1 A 200 V, 1.5 A	50 V, 0.75 A 60 V, 1.0 A 100 V, 1.6 A
	Without methanol:	Zeta-Probe	30 V, 0.1 A	60 V, 0.2 A	100 V, 0.43 A 150 V, 0.67 A 200 V, 0.91 A	15 V, 0.1 A	50 V, 0.55 A 100 V, 1.1 A 200 V, 1.5 A	50 V, 0.75 A 60 V, 1.0 A 100 V, 1.6 A
	10 mM $NaHCO_3$, 3 mM $NaCO_3$, pH 9.9 with 20% methanol:	Nitrocellulose	10 V, 0.1 A	20 V, 0.25 A	30 V, 0.44 A 45 V, 0.7 A 60 V, 1.0 A	5 V, 0.1 A	15 V, 0.43 A 35 V, 1.1 A 50 V, 1.6 A	20 V, 0.73 A 30 V, 1.1 A 40 V, 1.6 A
	Without methanol:	Zeta-Probe	10 V, 0.1 A	20 V, 0.25 A	30 V, 0.44 A 45 V, 0.7 A 60 V, 1.0 A	5 V, 0.1 A	15 V, 0.43 A 35 V, 1.1 A 50 V, 1.6 A	20 V, 0.73 A 30 V, 1.1 A 40 V, 1.6 A
Native gels acidic or neutral proteins	25 mM Tris, pH 8.3, 192 mM glycine without methanol:	Nitrocellulose Zeta-Probe	30 V, 0.1 A	60 V, 0.21 A	100 V, 0.36 A 150 V, 0.55 A 200 V, 0.85 A	15 V, 0.1 A	50 V, 0.5 A 100 V, 1.0 A 150 V, 1.5 A	50 V, 0.7 A 60 V, 0.9 A 100 V, 1.5 A
Isoelectric focusing native gels, basic proteins,acid urea gels	0.7% acetic acid	Nitrocellulose Zeta-Probe	30 V, 0.2 A	70 V, 0.5 A	100 V, 0.55 A 150 V, 0.85 A	15 V, 0.2 A	40 V, 0.60 A 70 V, 1.0 A	30 V, 0.60 A 60 V, 1.0 A
DNA, RNA	20 mM Tris, pH 7.8, 10 mM sodium acetate 0.5 mM EDTA	Zeta-Probe	30 V, 0.2 A	60 V, 0.6 A	80 V, 0.83 A 100 V, 1.03 A	15 V, 0.2 A	20 V, 0.8 A 35 V, 1.5 A	15 V, 0.80 A 25 V, 1.4 A
	50 mM Tris, pH 8.3, 50 mM sodium borate 1.0 mM EDTA	Zeta-Probe	30 V, 0.2 A	80 V, 0.55 A	100 V, 0.8 A 130 V, 1.0 A	15 V, 0.2 A	75 V, 0.8 A 100 V, 1.1 A	50 V, 0.75 A 90 V, 1.1 A

[illegible] All transfers should be performed at Constant Voltage setting. A super cooling coil must be used with the plate electrodes and for high intensity transfers.

4.3 Buffer Formulation

The following buffers are recommended for use with the Trans-Blot cell. For protein transfers, the Towbin[7] buffer provides efficient elution for most samples. The buffer systems of Bjerrum and Schaferd-Nielsen,[34] and Dunn[35] are at a higher pH and lower conductivity. These alternative buffers may provide increased transfer efficiency for proteins that are difficult to transfer.

1. [redacted] SDS-proteins using nitrocellulose (with methanol) or Zeta-Probe membrane (without methanol) 25 mM Tris, 192 mM glycine (20% methanol), pH 8.3. Dissolve 3.03 g Tris and 14.4 g glycine in 500 ml dd H_2O (add 200 ml of methanol); adjust volume to 1 liter with dd H_2O. Pre-chill the buffer before use.

   DO NOT ADD ACID OR BASE TO ADJUST pH. The buffer will range from pH 8.1 to 8.5 depending on the quality of Tris, glycine, dd H_2O and 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 not correct, check whether the electrode is designed to function with Tris buffers. If the pH electrode works properly with Tris buffers, and the pH is below 8.0, remake the buffer.

2. SDS may be added to Buffer 1 to increase protein elution from the gel.
   25 mM Tris, 192 mM glycine (20% methanol), 0.05-0.1% SDS, pH 8.3
   Dissolve 3.03 g Tris, 14.4 g glycine and 5-10 ml of 10% SDS in 500 ml dd H_2O (add 200 ml of methanol); adjust volume to 1 liter with dd H_2O. Pre-chill the buffer before use.

   DO NOT ADD ACID OR BASE TO ADJUST pH.

   Note: Addition of SDS to the buffer increases the conductivity of the buffer, resulting in increased current, power, and heating during the run. SDS may also affect the antigenicity of some proteins, and reduce binding of proteins to nitrocellulose.

3. Bjerrum and Schafer-Nielsen transfer buffer SDS-proteins using nitrocellulose (with methanol) or Zeta-Probe membrane (without methanol)[34]
   48 mM Tris, 39 mM glycine, (20% methanol) pH 9.2
   Dissolve 5.82 g Tris and 2.93 g glycine in dd H_2O (add 200 ml of methanol); adjust volume to 1 liter with dd H_2O. Pre-chill the buffer before use.

   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)[35]
   10 mM $NaHCO_3$, 3 mM NA_2CO_3 (20% methanol), pH 9.9
   Dissolve 0.84 g $NaHCO_3$ and 318 g Na_2CO_3 (anhydrous) in dd H_2O (add 200 ml of methanol); adjust volume to 1 liter with dd H_2O. Pre-chill the buffer before use.

   DO NOT ADD ACID OR BASE TO ADJUST PH.

5. DNA transfer buffer for Zeta-Probe membrane[36]
   5x TBE stock (0.5 M Tris, 0.5 M boric acid, 10 mM EDTA, pH 8.3)
   Dissolve 60.5 g Tris, 30.9 g boric acid and 3.73 g EDTA in dd H_2O; adjust volume to 1 liter with dd H_2O. For the working solution, dilute to 0.5x TBE by adding 100 ml of 5x stock TBE to 900 ml of dd H_2O. Pre-chill the buffer before use.

   DO NOT ADD ACID OR BASE TO ADJUST PH.

6. DNA transfer buffer for Zeta-Probe membrane[36]
   20x TAE (0.8 M Tris, 0.4 M sodium acetate, 20 mM EDTA, pH 7.8)
   Dissolve 96.9 g Tris, 32 g sodium acetate and 7.45 g EDTA in dd H_2O; pH to 7.4 with concentrated glacial acetic acid. Adjust volume to 1 liter with dd H_2O. For the working solution, dilute to 0.5x by adding 25 ml of 20x TAE to 975 ml of dd H_2O. Pre-chill the buffer before use.

   DO NOT ADJUST THE pH WITH HCl

7. 0.7% acetic acid for transfer of native gels[7]
   Add 7 ml concentrated glacial acetic acid to 993 ml dd H_2O. Pre-chill the buffer before use.

Section 5
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.

5.1 SDS-Protein Blotting

Standard Blot to Nitrocellulose

1. Equilibrate the gel in 500 ml of Towbin transfer buffer (Section 4.2) for 15 minutes.
2. Pre-chill buffer prior to transfer.
3. Assemble the sandwich as described in Section 3. Place into the middle slot of the tank and add 2.5-3.0 L of buffer.
4. For **standard electrode cards, transfer for 3 hours at 70 V (~0.25 A). Cooling is not required.**
5. For the **plate electrodes, transfer for 30 minutes to 1 hour at 50 V (~0.5 A). Cool to 4 °C with the Super Cooling Coil and a refrigerated recirculator.**

High Intensity Transfer

1. Follow steps 1 and 2, as above.
2. Assemble the sandwich and insert it into the slot closest to the cathode, as described under the high field intensity instructions, Section 3.4.
3. For standard electrode cards, transfer or 1 to 2 hours at 100 V (~0.36 A). Cool to 4 °C with the Super Cooling Coil and a refrigerated recirculator.
4. For plate electrodes, transfer 15-30 minutes at 100 V (~1.0 A). Cool to 4 °C with the Super Cooling Coil and a refrigerated recirculator.