

SEMI-DRY TRANSFER PROTOCOL

In semi-dry blotting the electrodes are placed directly in contact with the gel/nitrocellulose membrane sandwich to provide a fast, efficient transfer. The polyacrylamide gels must be equilibrated in transfer buffer, to remove electrophoresis buffer salts and detergents, and the nitrocellulose membranes and filter papers are pre-wetted, but that is all the buffer that is required (hence the term “semi-dry”). Using a platinum-coated titanium plate as the anode and a stainless-steel plate as the cathode, the Trans-Blot SD cell transfers in a horizontal configuration without a buffer tank or gel cassettes. Because of this direct contact there is a minimum of transfer buffer required (less than 200ml). It is important to exclude excess moisture and air bubbles trapped in the filter papers and membrane when setting up the transfer, usually a pipet rolled over the surface will take care of this, but other than that, the set-up for this process is extremely simple. Up to four mini gels can be transferred at the same time by placing them side-by-side on the anode platform.

Reagents

Semi-dry transfer buffer

1 liter:
5.82g Trizma Base
2.93g glycine
200 ml methanol
up to 1 liter w/dH₂O

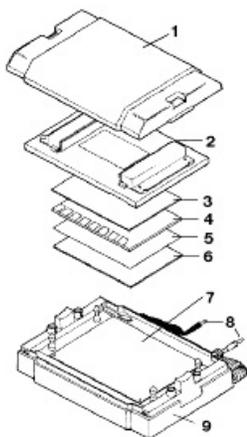
Materials

nitrocellulose: 1 piece 6x9cm for each gel
Schleicher & Schuell BA85 (10402580) 0.45µm

blot paper: 4 pieces 6x9cm for each gel
Schleicher & Schuell GB003 0.8mm

small containers to soak filter paper & gel

BioRad Semi-Dry Trans-Blot Cell



The Trans-Blot SD Semi-Dry cell:

1. safety lid
2. cathode assembly with latches
3. 2 pieces blot filter paper (S&S GB003)
4. gel
5. Nitrocellulose membrane
6. 2 pieces blot filter paper (S&S GB003)
7. spring-loaded anode platform, mounted on four guide posts
8. power cables
9. base

1. Prepare in advance the nitrocellulose and filter/blot paper (cut to the dimensions of the gel). For BioRad minigels:
 - 1 piece of nitrocellulose 6x9 cm
 - 4 pieces of blot paper 6x9 cm

Wet the membrane and allow it to soak 15-30 minutes. Complete wetting of the membrane is important to insure proper binding of proteins. Always use forceps or wear

- gloves when handling the membrane as it has a high affinity for protein binding and is very susceptible to scratches. *One helpful hint before wetting the nitrocellulose:* it's helpful to make a small pencil or ball-point pen mark on the top right-hand side of the nitrocellulose (a marker will smear across the filter when it gets wet). This is useful as a guide as to what is top/bottom, the date, the gel info, etc.
2. After running an SDS/PAGE gel, immediately equilibrate the gel in a small container of Semi-dry transfer buffer for ~15min. Equilibration helps facilitate 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, gels lower than 12% acrylamide will shrink in methanol-containing buffers. Equilibration allows the gel to adjust to its final size prior to electrophoretic transfer. Low molecular weight molecules (<10,000 daltons) may diffuse out of gels more readily; to limit this the equilibration time can be reduced to ~5 min.
 3. Remove the safety cover and the stainless the cathode assembly.
 4. Completely saturate a piece of blot paper by soaking in transfer buffer. Place this pre-soaked sheet of blot paper onto the platinum anode. Roll a pipet or test tube over the surface of the paper (like a rolling pin) to exclude all air bubbles. Repeat with a second piece of blot paper and place directly on top of the first piece.
 5. Place the pre-wetted nitrocellulose membrane on top of the wetted blot paper. Roll out the air bubbles.
 6. Carefully place the equilibrated gel on top of the nitrocellulose membrane, aligning the stack as perfect as possible. Transfer will be incomplete if any portion of the gel is outside the blotting area.
 7. Soak another piece of blot paper and place on top of the gel, carefully removing air bubbles from between the gel and filter paper. Repeat with a second piece of blot paper and place directly on top of the first piece.
 8. Carefully place the cathode plate onto the stack. Press to engage the latches with the guide posts without disturbing the gel/nitrocellulose stack. Place safety cover on the unit.
 9. Run the transfer unit at 400mA for 1 hour.
 10. Transfer efficiency can be checked by placing nitrocellulose filter in a small container and covering with Ponceau S dye (Sigma P-7170). Within a few minutes proteins should be able to be detected. Ponceau S dye can be reused (pour back into a conical tube) and filter can be rinsed gently with dH₂O tap water. Additionally, quality of proteins may be checked by Coomassie staining the gel after transfer as sufficient amounts of protein remain on the gel after transfer.

Comments:

- Transfers may not always be quantitative. A certain quantity of protein may be transferred through the membrane and onto the filter paper below.

- Nitrocellulose membranes can be used extensively for protein binding and detection. They can easily be stained for total protein by Ponceau S dye. Nitrocellulose has a high binding capacity of 80-100 μ g/cm². Low molecular weight proteins (<20,000 daltons) may be lost during post transfer washes, however, use of gluteraldehyde fixation and a smaller pore size nitrocellulose (0.2 μ m) have been shown to be effective in eliminating this loss¹.

References:

BioRad Trans-Blot SD Semi-Dry Electrophoretic Cell Instruction Manual

1. Polvino, W.J., Saravis, C.A., Sampson, C.E., and Cook, R.B., *Electrophoresis*, **4** 368 (1983).