Revised: February 4, 1999

**Electrotransfer of Proteins from Polyacrylamide Gels to Nitrocellulose Membranes.**

References:


- Bollag, D. M. and S. J. Edelstein, 1991. Protein Methods. Wiley-Liss, Inc., New York, NY.
- Instruction Manual, Mini-V 8-10 Vertical Gel Electrophoresis system. Life Technologies.
- Instruction Manual, Mini-Trans-Blot Electrode module. BioRad.


Materials:

Transfer Buffer
14.5 g Tris base
67 g glycine
1.2 L methanol
$dH_2O$ to 6 L
Store at 4°C.

$dH_2O$ in the cooling unit, stores at -70°C (BioRad apparatus only)

Immobilon-P Transfer Membranes (Millipore)

Protocol:

*Preparation:*

1. Cut a piece of the Immobilon-P to the size of the gel for each of the gels to be transferred. Cut of a corner of the membrane to mark the orientation. Use a pencil to label the membrane with your initials and date.
2. Cut two sheets of 3MM paper to the size of the fiber pads for each gel to be transferred.
3. Wet the Immobilon-P in 100% methanol for 15 seconds

   **Note**: Methanol is a P-listed chemical. The used methanol must be collected.
4. Transfer the Immobilon-P to distilled, deionized water for two minutes
5. Equilibrate the Immobilon-P and the fiber pads in transfer buffer for at least 5 minutes.
6. Soak the gels in transfer buffer for at least 15 minutes for a 0.75 mm gel. Proportionally increase the soaking time for thicker gels.
7. Rinse the buffer chamber thoroughly with $dH_2O$.

*Assembly of the polyacrylamide gel and membrane sandwich:*

1. Open the transfer cassette in a pyrex dish containing 1 - 2 cm transfer buffer. Place one of the soaked fiber pads onto the transfer cassette [black side of the BioRad transfer cassette or the Blot restrainer of the Life Technology apparatus (the small part)].
2. Wet a piece of filter paper. Place the gel onto the wet filter paper. Put the filter paper and gel onto the fiber pad in the transfer cassette.
3. Lay the presoaked Immobilon-P membrane over the gel. Gently roll a test tube over the Immobilon-P membrane to remove any air bubbles and make sure the membrane and the gel are in very close contact. They will almost stick together.
4. Wet the second piece of filter paper and lay it over the Immobilon-P membrane. Gently roll a test tube over the filter paper to remove any air bubbles.
5. Cover the filter paper with the second soaked fiber pad.
6. Close the transfer cassette. Place it into the buffer tank.

For the BioRad apparatus, repeat steps 1-6 for each gel (1 gel per cassette).

For the Life Technologies apparatus, repeat steps 2-5 for a second gel (2 gels per cassette).

*Electrotransfer:*

BioRad apparatus.

1. Put a small, thin stir bar into the apparatus and place the frozen cooling unit into the apparatus.
2. Place the apparatus onto a magnetic stir plate and begin stirring.
3. Fill the tank with transfer buffer.
4. Attach the electrodes.
5. Set the power supply to 100 V, constant voltage, and transfer for 1 hour. Alternatively set the voltage at 30 V for overnight transfers.

Life Technology apparatus.

1. Fill the tank with transfer buffer.
2. Attach the electrodes.
3. Set the power supply to 150 V, constant voltage, and transfer for 1 hour.

When the transfer is complete, turn off the power, disconnect the leads and remove the lid of the tranfer assembly. Remove the transfer cassette. Disassemble the cassette.

Discard the filter paper and the gel. Place the blotted membrane, protein side up on 3MM filter paper. Air dry the blotted membrane for 2 hours at room temperature, or 1 hour at 37°C.

Storage: Deal the blot in a plastic bag. Place the bag between two sheets of cardboard. Label the cardboard with the name of the blot, your initials and the date. Store the assembly at -20°C to prevent oxidation. The cardboard protects the membrane from breaks or cracks. Allow the membrane to thaw to ambient temperature before removing the cardboard.

**Note:** Methanol is a P-listed chemical. The used transfer buffer must be collected.