**Electrophoretic Transfers**

**Semi-Dry Blotting Procedure**

1. Soak the filter paper in transfer buffer (two sheets of extra thick or six sheets of thick filter paper).
2. Remove the safety cover and stainless-steel cathode assembly and place the presoaked filter paper (one sheet of extra thick or three sheets of thick paper) onto the platinum anode. Remove air trapped between the paper and the anode using a blot roller.
3. Carefully place the equilibrated membrane on top of the filter paper. Roll out any air trapped between the transfer materials.
4. Gently place the equilibrated gel on top of the membrane and roll out trapped air.
5. Place filter paper (one sheet of extra thick or three sheets of thick) onto the gel and roll out trapped air.
6. Carefully place the cathode assembly onto the transfer stack and then place the safety cover back onto the unit.
7. Connect the cables to the power supply, making sure to match the colors on the cables to those on the power supply inputs. Program the power supply (see Chapter 4) and start the run.
8. Upon completion of the run, remove the cathode assembly and disassemble the gel and membrane sandwich. If needed, rinse the gel briefly with diH₂O.

**TIPS**

- Evaluate transfer efficiency at various field strengths (V/cm), staying within the recommendations for each instrument.
- Bio-Rad semi-dry systems place the anode on the bottom electrode. If using a different system, consult the owner’s manual for the proper orientation of the gel and membrane.
- For transfers using high power, monitor the transfer carefully and use cooling as needed.
- Perform a test run to determine the time required for complete transfer. Times may vary from 15 min to 1 hr, depending on many factors, including the power setting, and the size, shape, and charge of the protein.
Trans-Blot Turbo Blotting Procedure

1. After gel electrophoresis, open the transfer pack that matches your gel (mini or midi) and place the anode stack on the cassette base. Place single mini or midi stacks in the middle of the cassette base; two mini gels can be placed on a midi stack with each gel bottom facing the center. Use the blot roller to remove any air trapped between the pad and membrane. No equilibration is required.

2. Place the gel on the anode stack (which includes the membrane) and the cathode stack on the gel. Roll to remove trapped air.

3. Place the lid on the cassette and lock it into place by turning the green knob clockwise. Ensure the locking pins fully engage their locking slots.

4. Turn the instrument on and slide the cassette into either cassette bay. If using two cassettes, each must be using the same size transfer pack.

5. Start the transfer. With the cassette inserted into the instrument, press TURBO and select the gel type. Press A:RUN to start the top tray, B:RUN for the bottom tray. Select LIST to select a preprogrammed protocol or NEW to create and run a new protocol.

6. At the end of the run, RUN COMPLETE appears on the screen. Remove the cassette from the instrument and unlock the lid. (Caution: the cassette may be warm.) Remove the membrane from the transfer sandwich and discard the remaining transfer pack materials.
This is an excerpt from Bio-Rad's comprehensive Protein Blotting Guide (Bulletin 2895).