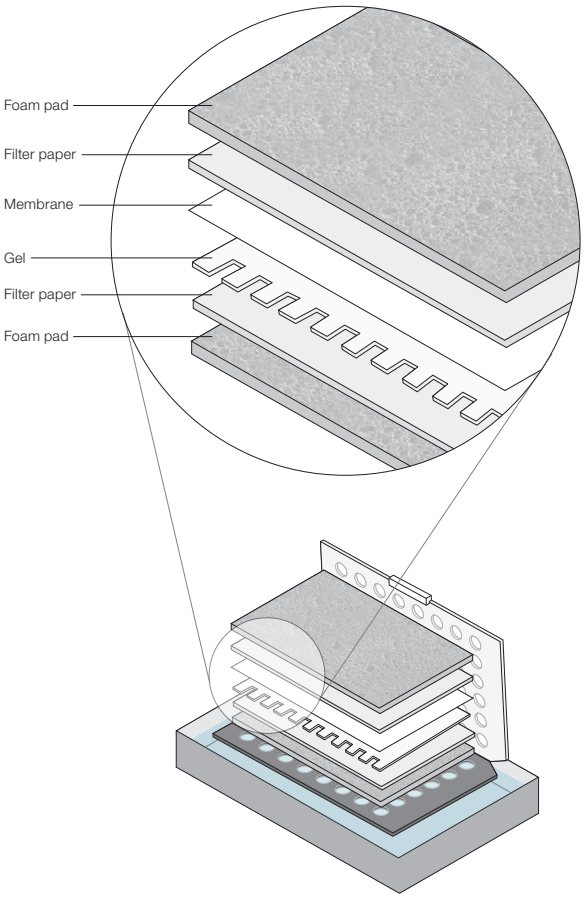
Electrophoretic Transfers

Protocol

Bulletin 6213

Tank Blotting Procedure Part I

Prepare the Gell and Membrane Sandwich



Images and material shown are based on Bio-Rad tank blotting products. Materials may differ based on your blotting apparatus manufacturer.

1. Open a gel holder cassette and submerge the cathode (black) side in transfer buffer.
2. Wet a foam pad in transfer buffer and place it on the submerged side of the cassette.
3. Wet a piece of filter paper in transfer buffer and place it on top of the foam pad. Use a blot roller to remove trapped air.
4. Place the equilibrated gel on top of the filter paper. If needed, gently use a blot roller to remove trapped air.
5. Place the equilibrated membrane on top of the gel. Use a blot roller to remove trapped air.
6. Wet a second piece of filter paper in transfer buffer and place it on top of the membrane. Again, roll to remove trapped air.
7. Soak a foam pad in transfer buffer and place it on top of the filter paper, then close and lock the cassette.

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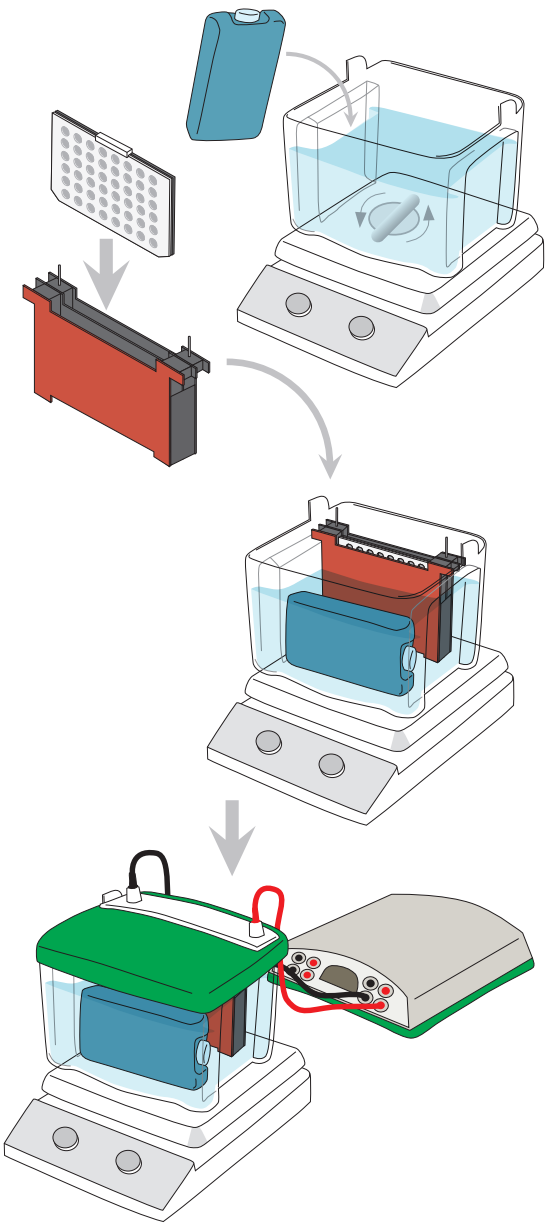
Tank Blotting Procedure Part II

Assemble the Tank and Program the Power Supply



1. Place the transfer tank on a magnetic stir plate and fill the tank halfway with transfer buffer.
2. Add a stir bar and begin stirring. If needed, begin cooling the transfer tank with an ice pack or cooling coil.
3. Insert the gel holder cassette into the blotting module latch side up, with the black side of the cassette facing the black side of the blotting module. Repeat with additional casettes if needed. Place blotting module with cassettes in the tank.
4. Add transfer buffer to the tank until the buffer level reaches the fill line.
5. Place the lid on top of the cell, making sure that the color-coded cables on the lid are attached to the proper electrode cards.
6. 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 and start the run.

   Note: Reversing field polarity by switching cable colors will cause irreversible damage to the electrodes.
7. Upon completion of the run, remove the cassettes and disassemble the gel and membrane sandwich.

TIPS

Stirring during transfer helps maintain uniform conductivity and temperature. Failure to properly control buffer temperature may result in poor transfer and poses a potential safety hazard.

Electrophoretic transfer entails large power loads and consequently, heat generation. The tanks are effective thermal insulators and limit the efficient dissipation of heat; thus, simply placing the tank in the cold room is not enough to remove all of the heat generated during transfer.

Effective cooling required for high-intensity field transfers and recommended for long, unsupervised runs can be provided using the cooling coil or Bio-Ice™ units included with your transfer device.

Evaluate transfer efficiency at various field strengths (V/cm), staying within the recommendations for each instrument.

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 overnight, depending on many factors, including the power setting, gel percentage, and the size, shape, and charge of the protein.

This is an excerpt from Bio-Rad's comprehensive Protein Blotting Guide (Bulletin 2895).

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