

Semi-Dry Transfer of Proteins for Western Blotting

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The semi-dry blotting transfer uses flat-plate electrodes, a platinum-coated titanium plate as the anode and a stainless-steel plate as the cathode, to drive force of an electric field eluting the proteins from the gels and immobilize them on a nitrocellulose or polyvinylidene difluoride (PVDF) membrane. The configuration is horizontal without a buffer tank or gel cassettes. As a result, a minimal amount of buffer is required. In addition, mini-gels can be placing side-by-side to allow transfer of up to four gels at the same time. This method is fast, efficient, and maintains the high-resolution protein pattern.

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Equipment

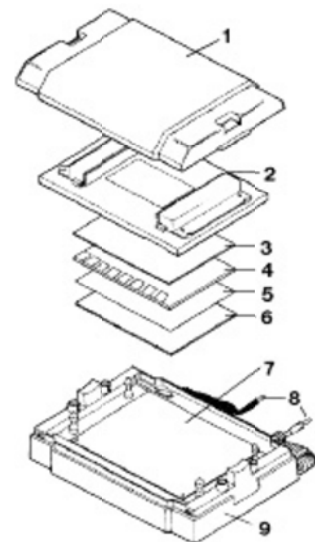
- PVDF (preferred) or nitrocellulose membrane: 1 6 x 9cm piece for each gel (0.45 µm)
- Blot paper: 4 pieces 6 x 9 cm for each gel (0.8 mm)
- A semi-dry blotting apparatus. We use the BioRad Semi-Dry Trans-Blot Cell

Buffers

- **Anode I Buffer**
 Weigh 3.64 g Tris base and dissolve in 70 ml ddH₂O
 Adjust pH to 10.4
 Add 10 ml methanol
 Bring the solution to 100 ml with ddH₂O
- **Anode II Buffer**
 Weigh 0.303 g Tris base and dissolve in 70 ml ddH₂O
 Adjust pH to 10.4
 Add 10 ml methanol
 Bring the solution to 100 ml with ddH₂O
- **Cathode Buffer**
 Weigh 3.0285 g Tris Base and 5.25g 6-aminocaproic acid and add to 600 ml ddH₂O
 Adjust pH to 9.4
 Add 100 ml methanol
 Bring the solution to 1 L with ddH₂O

Assembly of Transfer Apparatus

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



Methods

1. Prepare in advance the PVDF or nitrocellulose membrane and filter/blot paper (cut to the dimensions of the gel). To activate the PVDF, soak in methanol for 5 seconds and then wash one time in water. Activation of the membrane is important to insure proper binding of proteins. The filter paper should be soaked in transfer buffer for at least 20 minutes before transfer.
2. Equilibrate the gel after running SDS/PAGE gel for 15 min in Anode II buffer. This will help the removal of salts and detergents present in the electrophoresis buffer. The conductivity of the transfer buffer and the amount of heat generated during the transfer can be affected by salts and detergents. Acrylamide gels can shrink in presence of methanol so presoak the acrylamide gels in methanol-containing buffers allows the gel to adjust to its final size.
3. Remove the safety cover and the stainless the cathode assembly.
4. Place two blot paper sheets presoaked with Anode I buffer onto the platinum anode. Use a pipet to roll over the surface of the paper to remove air bubbles. Place one blot paper sheet presoaked with Anode II buffer on top of the other two and use the same procedure to remove bubbles.
5. Place the nitrocellulose membrane on top of the blot paper. Remove the air bubbles.
6. Place the gel on top of the nitrocellulose membrane, carefully aligning the sandwich.
7. Place three pieces of blot paper sheet presoaked with cathode buffer and place on top of the gel, carefully removing air bubbles from between the gel and filter papers.
8. Place the cathode plate onto the sandwich and place safety cover on the unit.
9. Run the transfer unit at 1 mA for for cm^3 for 1 hour.
10. Transfer efficiency can be checked by placing nitrocellulose filter in a small container and covering with 0.1% Ponceau S dye solution (Sigma P-7170). Stain the gel with Coomassie [1% (w/v) in 45% (v/v) methanol and 10% (v/v) acetic acid] after transfer to determine the amounts of protein that remain on the gel after transfer.

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