Stamatatos Lab
Western Blotting

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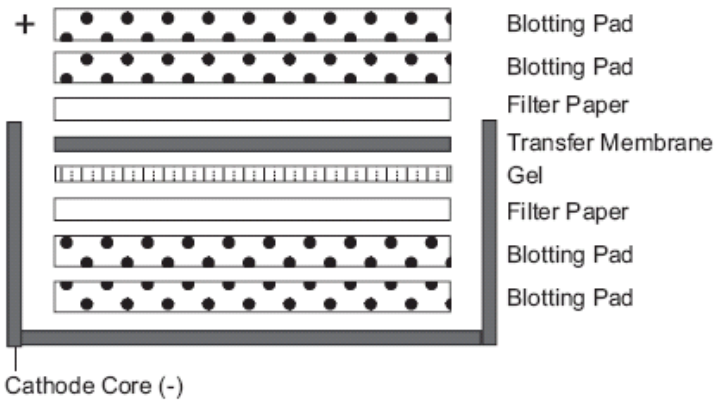
Transferring to membrane

1. Pre-soak blotting pads in transfer buffer (+ 10% methanol)
2. Cut filter paper and PVDF membrane to appropriate size.
3. Soak PVDF membrane in methanol for a few minutes, then rinse in dH2O, and soak in transfer buffer.
4. Set up XCell transfer apparatus as shown below, ensuring no air bubbles are trapped between the filter paper, the gel and the membrane.

Set up for transferring one gel:



5. Place transfer apparatus in electrophoresis box and lock in place.
6. Fill inner chamber with 1x transfer buffer w/ 10% methanol until the blotting pads are just covered (approx 50-60ml). Fill the outer chamber ¾ full with dH2O.
7. Transfer can be done on the bench at room temp at 20-30V for approx 1 hour, or overnight at 5-10 V. Transfer conditions are membrane and gel dependent: see table below.

Type of the Gel	Transfer Buffer (1X)	Membrane	Transfer Conditions	Expected Current
Novex® Tris-Glycine Novex® Tricine	Novex® Tris Glycine Transfer Buffer with 20% methanol. 1X Transfer Buffer should be pH 8.3 before addition of SDS or methanol. Do not adjust the pH.	Nitrocellulose or PVDF	25 V constant for 1-2 hours	Start: 100 mA
NuPAGE® Bis-Tris	NuPAGE® Transfer Buffer with 10% methanol for transfer of one gel. NuPAGE® Antioxidant for reduced samples	Nitrocellulose or PVDF	30 V constant for 1 hour	Start: 170 mA End: 110 mA
NuPAGE® Tris-Acetate	NuPAGE® Transfer Buffer with 10% methanol for transfer of one gel. NuPAGE® Antioxidant for reduced samples	Nitrocellulose or PVDF	30 V constant for 1 hour	Start: 220 mA End: 180 mA

Blotting

1. Remove membrane from chamber and block at room temperature with rocking for approx 1 hour. (Block buffer: 5% milk in PBS/ 0.6% Tween. Store at 4C.)
2. Add primary antibody (in block buffer) and incubate overnight at 4C with rocking.
3. Next day, remove primary Ab.
4. Wash membrane 3-5 times (10min each wash) at room temperature with shaking. (Wash buffer: PBS/0.6% Tween)
5. Add HRP-conjugated secondary antibody in diluted appropriately in blocking buffer. Protein G HRP = 1:1000
   Goat anti-human = 1:3000
   Goat anti-mouse = 1:5000
6. Incubate at room temperature with rocking for approx 1 hour.
7. Discard secondary and wash membrane.
8. Add substrate, then place membrane in cassette and expose to film in darkroom.