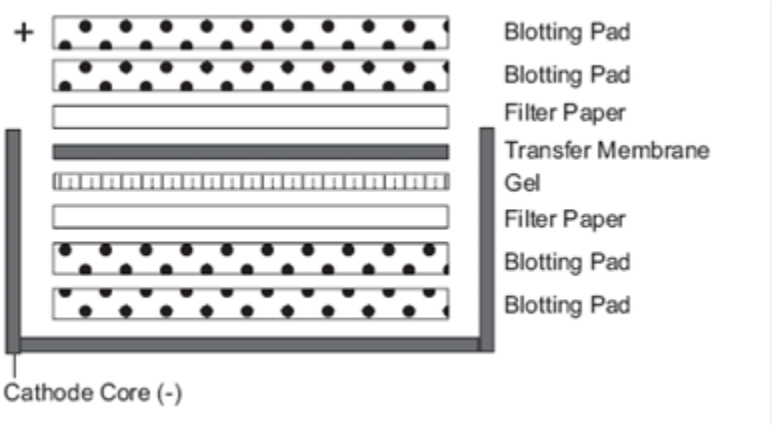
Western Blotting

Stamatatos Lab

Contact: Andrew Stuart (astuart@fredhutch.org)

Components needed: XCell II Blot Module, Novex Mini-cell electrophoresis box, PVDF or nitrocellulose membrane, Whatman paper, blotting pads, transfer solution/buffers (specific to membranes/gel).

1. Separate proteins by SDS PAGE, remove gel and equilibrate in transfer buffer for 15min.
2. Pre-soak blotting pads in transfer buffer.
3. Cut filter paper and PVDF membrane to appropriate size.
4. Soak PVDF membrane in methanol for 15 seconds, followed by water rinse, then soak in transfer buffer (note: do not soak nitrocellulose in methanol, instead place directly into transfer buffer instead).
5. Set up the blot module as shown below, ensuring no air bubbles are trapped between the filter paper, gel and membrane.



6. Place blot module into the electrophoresis box and lock in place.
7. Fill blot module with transfer buffer until the blotting pads are covered. Fill the outer chamber ¾ full with ice and then fill with water.
8. Generally transfers can be completed on the bench at room temperature at 65V for 1 hour (see membrane and gel recommendations for conditions).
9. Remove membrane from chamber, rinse in wash buffer, then block at room temperature with rocking for 1 hour.
10. Add appropriate amount of primary antibody and incubate at room temperature with rocking for 1 hour.
11. Remove primary antibody and wash membrane at room temperature with rocking for 5 minutes (repeat for a total of 3 washes).
12. Add appropriate amount of HRP-conjugated secondary antibody and incubate at room temperature with rocking for 1 hour.
13. Discard secondary and wash membrane as in step 11.
14. Add substrate, place membrane in cassette and expose to film.