

Western Blotting Tips

- 1) Load 20 to 35 μ l of your samples unless you know for a fact that this is too much.
- 2) In the first lane load 25 μ l of positive blotting control. This is lysate stimulated with PMA or LPA. This is good for most CCL39 blots.
- 3) Molecular Wt Markers - There are now two kinds. One is the BioRad Prestained standards we've always used. The other is MagicMarker. This is a non-stained protein that has an IgG recognition site. That means each of the proteins will bind secondary antibody and will be visible on the film. To use this:
 - In the second lane load 10-15 μ l of the BioRad standard
 - In the SAME well/lane add 3 μ l of the MagicMarker standard (found in a box in the freezer in the main room)
- 4) Run the gel to the bottom but not off. Make certain the dye is just coming to the edge of the glass not the gasket. Remember, 200 v for 1 hour is an approximation - you may very likely have to turn it up a little more. If this is a key experiment, run the gel at 100 v for 10-15 min first then run for 200V. The proteins will be tighter. ALSO if the % cross-linking is low then the proteins will run faster than the higher % gels
- 5) Soak the gel in transfer buffer for a minute or two to reduce salt that can reduce transfer.
- 6) Cut the paper to fit the gel, unless you are using ERK or p-ERK. Remember unless we have done the blot many times you need to show the molecular weights to prove that the band you think is your protein is the right one. Clip the corner of the blot as directed in the protocol. Not all antibodies are as specific as some of the ones we use. Always mark the film with the markers!
- 7) The fit of the gel sandwich should be tight and free of air bubbles. If the pads are getting thin add a third to make certain there is proper contact between paper and gel.
- 8) Transfer at 90 V for 1 hr unless using a 12-16% gel then add 15 min
- 9) Some antibodies need a different washing solution. The concentration or type of detergent (Tween-20) may not work for some. If using a new antibody, check!
- 10) Block in the BioRad dry milk, not the carnation or other generic instant milk. Use the same concentration as before. We get high backgrounds with some of these other milks. Some antibodies need BSA instead of milk to block with. Always look up new antibody requirements.
- 11) When starting use 1:500 dilutions for the primary and 1:1000 for the secondary. Use only the antibodies from the "Chinese take-out" cool-safe box
- 12) Don't let the blot get too dry - this may have been a problem
- 13) Do a 10 sec, 1 min and 10 min exposure.

Pray hard, live well and good luck