**Western Blot – Invitrogen supplies**

1. Prepare samples
   1. Run BCA to determine total protein concentration in each sample
   2. Make 40µl total (gels with 10 wells hold 37µl) by mixing 8µl of 5x sample buffer, and the appropriate amount of diH₂O and sample to get the same total protein concentration in each well.
   3. Boil for 5 min at 100°C
2. Remove comb and tape from gel and rinse with diH₂O. Place 2 gels, or 1 gel and the buffer dam, into the gel box, lock into place.
3. Fill the space between the 2 gels with1x running buffer and make sure it isn’t leaking. Then fill then rest of the box with running buffer.
4. Load 5-10µl of ladder into the appropriate lane, and 37µl of sample into each other lane. If you have extra lanes, load 2-5µl of sample buffer.
5. Run on Tris-Glycine setting. This runs for 1.5 hours, but if the loading dye band is not near the bottom of the gel, run longer until it gets there.
6. As soon as the gel is done, begin transfer so the protein doesn’t diffuse out of the gel.
7. Pour transfer buffer (100ml 10x transfer buffer, 200ml methanol, 700ml diH₂O) into a bucket that you can use to build the sandwich. Put sponges and filter paper into the buffer.
8. Break the casing around the gel with a small flathead screwdriver or the Invitrogen tool.
9. Build the sandwich in the transfer buffer as follows (cathode is on the bottom, anode on the top) then place it into the cathode with the anode on top:

**For one gel:**

Sponge

Sponge

Filter paper

Membrane

Gel

Filter paper

Sponge

Sponge

**For two gels:**

Sponge

Filter paper

Membrane

Gel

Filter paper

Sponge

Filter paper

Membrane

Gel

Filter paper

Sponge

1. Place whole set up (pressed between the anode and cathode) into the gel box and secure. Fill with transfer buffer from the bucket used to build the sandwich. Place the lid on top and run on western blot setting. This will run for 1.5 hours.
2. Disassemble the transfer set up, and wash the membrane in PBS or TBS for 2 minutes.
3. Block in abdil for 1-2 hours at room temperature, while shaking at 30-50 RPM.
4. Stain in appropriate dilution of primary antibody diluted in abdil overnight at 4°C.
5. Wash 3x for 10 min in TBS-T, while shaking at room temperature.
6. Stain in appropriate dilution of secondary antibody diluted in abdil at room temp for 1 hour, covered. It should be shaking at 30-50 RPM.
7. Wash 3x for 10 min in TBS-T, shaking at room temp.
8. Rinse membrane once in PBS or TBS.
9. Image with Licor imager (at 680nm for current 2ndary antibody)