Western Blot

Set up the casting chamber
- set gray foam on bottom ledge
- Rinse glass plate and spacer w/ 70% ethanol (choose between .75mm and 1mm)
- Line up plates so that “Bio-Rad” is exposed
- Open green gates by doing what actually feels like closing them
- Slide plates into grooves – right side up as marked
- Press down firmly onto foam
- Close/clamp gates (push away from plates)
- Pull pressure lever down onto plates

Prepare your gel according to chosen recipe (sample below). This is written for a stacking gel. If you are doing a gradient gel, just buy one from the Bio-Rad fridge upstairs.

5ml 12% acrylamide (neurotoxin – wear gloves)

1.7mL H20
2.0mL 30% acrylamide solution
1.3mL 1.5M pH 8.8 Tris
.05 10% SDS
.05mL 10% APS (ammonium persulfate)
.002 TEMED (stinky)

Add last two ingredients just when ready to pour gel. Pipet completed gel from 1mL pipet in between plates. Fill to bottom of green ledge.

Add 100ul of ddH2O to each side of gel. It will spread itself out.

Let polymerize (there will be extra gel left in your tube. You can observe this to know when your gel is polymerized). When solid slip a piece of filter paper in to absorb water. Prepare stacking gel (example below)

4 mL Stacking gel (Will really only use 2mL of it)

2.7mL H20
.67 30% acrylamide solution
.5mL 1.0M Tris ph 6.8
.04mL 10% SDS
.04mL 10 APS
.004mL TEMED
Again, leave out the last two until really ready to pour. Pipet stacking gel in between plates right on top of previous gel. Fill to top and gently insert comb. Some will squish out – just wipe it up. Let polymerize.

Prepare samples in the mean time. Typically you want to load 25μg of protein for each sample. Use the concentrations you determined earlier to find what volume of sample you need. Add 5μl of completed Laemneli’s Sample Buffer (below) to each sample. Add 5μl of the same to 5μl of the Kaleidoscope markers (5μl aliquots in –20 freezer). Poke hole in top (unless radioactive) and incubate at 100 C for 5 mins.

In order to load the gel you must remove it from the casting tray.
- Release the top pressure lever, open gates and slide plates/gel out the top.
- Slide plates into gel box, gel facing inwards.
- Slide this into the larger chamber, pushing down and close gate.
- Slide this conglomeration into the tank.

Fill inner chamber with SDS buffer (recipe below) up to top. Check for leaks. If no leaks fill outer chamber just so that the bottom of the plates are submersed. If the inner chamber is leaking fill out chamber completely full. Ensure that there are no bubbles against the gel.

Run @ 40mAmps until dye front reaches very bottom of the gel.

Laemneli’s Sample Buffer

120mm Tris pH 6.8
4% SDS
20% glycerol
.002% BPB (Bromophenol blue) (or a little more if just not dark enough)

Add 5μl/ml betamercapto and 100mM DTT (100μl.ml of 1M stock) just prior to use.

10x SDS Running Buffer

250mMTris
1.9M Glycine
1% SDS

(To 800mL ddH2O add

30.3g Tris base
144.2g Glycine
10g SDS)

ph to 8.3 with HCl, bring to 1L with ddH2O. Dilute 1:10 with water