SDS-PAGE and Western Blot Protocol
11/23/11

Materials:

Laemmli sample buffer (Bio-Rad #161-0737)
10x Tris/glycine/SDS running buffer and transfer buffer (Bio-Rad #161-0772)
Mini-PROTEAN Tetra cell (Bio-Rad: see manual)
Mini-PROTEAN TGX gels (Bio-Rad)
Nitrocellulose membrane
Blotting paper
Chemiluminescent western blot substrate (Bio-Rad)

Procedure:

SDS-PAGE

- Assemble 2 ready gel precast gels into the tetra cell electrophoresis module, remember to remove the clear tape at the bottom of the Ready gel cassette
- Fill the Tetra Cell with 1x running buffer of choice (~700mL)
- Aliquot ~10-20ug of total protein to load per lane
- add laemmli sample buffer at 1:1 ratio and load to a ready gel using gel loading tips
- Run the gel at 200 V ~35mins
- Remove gel from the ready gel cassette
- Rinse gel with transfer buffer

TRANSFER

- Wet nitrocellulose membrane in 1x transfer buffer ~5 minutes
- Assemble sandwich as per instruction manual for the Mini-PROTEAN Tetra cell negative node side of cassette>sponge>blotting paper>gel>membrane>blotting paper>sponge>positive node side of cassette. Handle the membrane with tweezers
- Fill the Tetra Cell with 1x transfer buffer of choice (~1000ml)
- Transfer at 100 V ~1hr
- Remove membrane and dry until ready for western blotting or place directly into blocking buffer

Western Blotting

- Rock membrane with blocking buffer ~15 minutes
- Wash 3x with wash buffer ~5 minutes each in a shaker
- Incubate with primary antibody in ~10 mL of dilution buffer on a rocker for 1hr at RT or overnight at 4 degrees

- Wash 3x with wash buffer ~5 minutes each in a shaker
- Incubate with horseradish peroxidase (HRP) conjugated secondary antibody in ~10 mL of dilution buffer on a rocker for 1hr at RT
- Wash 3x with wash buffer ~5 minutes each in a shaker
- Mix chemiluminescent western blot substrate 1:1
- Place about 1 ml of this mixture onto a transparency and blots with antibody side facing down over the pool of mix.
- Wait 5 minutes, remove excess liquid by touching the edge of the blot to a wipe and expose on to film.

Buffers

Wash buffer (TBS/T)
20 mM Tris
150 mM NaCl
0.1 % Tween 20
5% non fat milk

Blocking buffer
1xTBST
5 % non fat milk

Dilution Buffer
1xTBST
5 % BSA