Western:

Protein gel-
We use either a self-poured acrylamide gel (for 15% or gradients not available commercially) or precast polyacrylamide gel.
A. Protocol for precast polyacrylamide mini gel:

1. Make 700ml 1xelectrophoresis buffer from 10x stock running buffer (A)
2. Remove igel from packet and mark bottom of wells. You should also number your gels if doing multiple gels.
3. Use the Protean III system; make surethe Bio-Rad gel running frame green gasket's flat side is facing outward
4. If running one gel use a blank cassette on one side of the frame and gel on other side. If running two gels put both the gels on to the running frame so that lower plate faces inside of the apparatus and lock in place.
5. Put the running frame into the tank.
6. Fill the inner chamber of tank with electrophoresis buffer. Make sure there are no leaks; if it does leak, remove the gels, wet the inside of the gel with buffer (the part facing the gasket) and lock the gel in place again. Fill the outside of the chamberwith running buffer so that the bottom of the sample wells level with the buffer
7. Prepare protein samples for loading using 15-50 ug of protein per sample. It's often helpful to prepare an Excel spread sheet with amounts of protein, H2O and 6X loading dye so that total volume is 45-50 ul per well.
8. Use x12 ul of marker
9. Heat the sample (but not the marker) for 3-5 mins at 90-100 deg C, put them on ice immediately.
10. Rinse the wells thoroughly with running buffer using a syringe and needle to remove air bubbles.

12. Load the samples avoiding spilling into adjacent wells

13. Run the gel at 100V (~60-80amps) for one hour. Gel run time will depend on the size of your protein—small proteins can run off low percent gels, so use the markers as a guide.

Transfer :

A. Preparation

1. While the gel is running cut the membrane (Immobilon-P) to the standard gel size – 8.5 X 5.5 cm/gel
2. Cut the Whatman paper to the same size as the membrane- (six sheets/gel)
3. Soak the membrane in 100% methanol for 15 seconds.
4. Transfer membrane to Milli-Q grade water and leave it for 2 mins
5. Equilibrate the membrane for at least 5 mins in the transfer buffer.
6. Once gel has completed it's run, open precast gel with razor blade or coin at corner. Remove wells and notch corner with razor blade .
7. Place gel in transfer buffer to equilibrate (15-30 min—longer time for thicker gels)

B. Transfer:

Buffers:

A. 10x electropheresis buffer:

Add recipe from methods book