Western Blotting for 1C2, 3B5H10, and 4C8 Antibodies

1. Begin western blot procedure by loading protein into SDS – polyacrylamide gel. On the whole, I normally use 8, 10, or 12% gels (depending on the size of protein you run in the gel).
2. Run gel at around 120 volts. I normally stop the gel running procedure when my leading blue dye band reaches very close to the bottom of the glass plates. The gel is run in 1:10 transfer buffer and H2O (1 liter), respectively.
3. I transfer the gel onto nitrocellulose blot paper and run the transfer at a 4ºC environment. I set the apparatus to 90 volts and run for 45 minutes. The buffer solution for the transfer (1 liter) is 7 parts H2O, 2 parts Methanol (CH3OH), and1 part 10x transfer buffer.
4. Once the transfer has been completed place nitrocellulose blotting paper (which should contain the protein following the transfer - a good indicator that your transfer is successful is evidence of the benchmark molecular weight marker on the paper), in a container and rinse at least 3x with 1xPBS.
5. Make up a solution of 1xPBS with 3% milk powder and incubate at 4ºC overnight.
6. Once block is complete give quick rinse with 1xPBS and make up Primary Ab solution. Primary Ab solution is 1xPBS with 0.3% milk powder. Add to this your desired antibody - 4C8 – 1:5000, 1C2 – 1:1000, and 3B5H10 – 1:15000. Incubate primary antibody at room temperature for 2 hours. This process should be done on a rocker or shaker mechanism.
7. After Primary Ab labeling is complete wash blot with 3X 1xPBS for at least 5 minutes each time.
8. Secondary antibody solution is the same as primary antibody solution with the exception of different antibody. We use a goat anti- mouse antibody (labeled HRP mouse) at 1:20,000 ratio. Incubate this solution for 2 hours on a shaker/rocker at room temperature.
9. Once you have completed this secondary antibody labeling rinse the blot in 1xPBS three times at room temperature.
10. The next step is to ECL stain the blot with a chemiluminescience kit and run the blot through a developer to detect our antibody labeled protein. I usually incubate the blot in the chemiluminescience mixture for 2 minutes. I then expose the blot to film in 10 second, one minute, and two minute increments.
11. Note: it is usually a good idea to go through this procedure with someone who has already gone through the protocol before venturing out on your own. You can then use this protocol as a guide.