Western Blot Protocol

1. Run SDS PAGE gel
2. Cut out PVDF membrane (also cut a corner to mark bottom and upper)
3. Activate PVDF membrane with methanol by incubating for 15 sec
4. Soak activated membrane in transfer buffer (1x Tris-glycine, 20% methanol), to equilibrate for 5 min
    a. Do not let the membrane dry out! If a white spot is visible, re-wet membrane in methanol first and then equilibrate in the transfer buffer
5. Rinse gel in transfer buffer, wet filter paper and pads in the transfer buffer
6. Assemble the sandwich in the transfer cassette as follows:
    a. White side of cassette (+)
    b. Sponge
    c. Whatmann filter paper (1 piece)
    d. PVDF membrane
    e. SDS Gel
    f. Whatman filter paper (1 piece)
    g. Sponge
    h. Black side of Cassette (-)
7. Insert the cassette into the slot with black to balck and white to red
8. Cover to just over top wire with transfer buffer after adding ice pack in the slot. Put the apparatus in the ice
9. Run at 100 V for 1-3 hours
10. Remove membrane from the sandwich. Do not allow to dry out
11. Block the membrane in blocking solution (1x PBS with 5% skim milk) for 1 hour at R/T with shake (or at 4 degrees overnight without shake)
12. Pour off blocking solution. Add primary antibody in blocking solution (1x PBS with 5% skim milk, 0.1ug/mL primary) and incubate for 1 hr with shake
13. Wash 3x with wash buffer (0.1% Tween in 1x PBS, a.k.a. PBST), 5 min each with shake
14. Add secondary antibody in blocking solution (1x PBS with 5% skim milk, 0.04ug/mL secondary antibody) and incubate for 1 hr
15. Wash 3x with wash buffer (0.1% Tween in 1x PBS, a.k.a. PBST), 5 min each with shake; additional wash with ddH2O with shake

16. Add ECL western blotting substrate: premix 1 mL of each solution (solutions 1 and 2 from ECL kit) in a falcon tube and add the mixed solution to the membrane by pipetting several times to make sure the membrane fully contacts with the solution. Incubate at R/T for 5 min.
17. Run protocol at BIO-RAD ChemiDoc™ station using protocol as follows:
    a. blots>>>Chemi>>>manually set exposure time: 10, 30, 50, ….300 second until you see the band