Western Protocol

1. Run protein gel (for Int this is usually a 4-12% TG gel at 125V for 2 hours)
2. Prepare transfer buffer:
    a. 7.2 g glycine
    b. 1.45 g tris-base
    c. 200 ml methanol
    d. fill to 1 liter with nano-pure water
3. Soak gel and all required apparatus for a few minutes in transfer buffer (assembly: negative plate (larger one), 2 blotting pads, filter paper, gel, nitrocellulose, filter paper, 3 blotting pads, positive plate).
4. Transfer proteins to nitrocellulose at 30 V for 2.5 hours on ice.
5. Prepare TBST
    a. 2 ml of 1M Tris-HCl pH 7.4
    b. 6 ml of 5M NaCl
    c. 2 ml of Tween 20
    d. 190 ml of nano-pure water
6. Block the nitrocellulose at room temperature in 10 ml of 5% milk dissolved in TBST (allow at least 30 minutes for milk to fully dissolve).
7. Remove 6 ml of block and add 6 ml of TBST. Add 50 µl of Int Ab.
8. Shake at room temperature for 5 minutes, then transfer to 4°C overnight. Alternatively, shake at room temperature for > 1 hour and proceed with step 10. (primarily for dot-blots)
9. Shake at room temperature for 1 hour
10. Wash off 1° Ab with 2 washes of 10 ml TBST for 5 minutes at room temperature
11. Add 5 ml block and 5 ml TBST and 1 µl of 2° Ab.
12. Shake at room temperature for 1 hour.
13. Wash off 2° Ab with 4 washes of 10 ml TBST for 5 minutes at room temperature.
14. Rinse with nano-pure water
15. Expose to substrate for 5 minutes.
16. Expose to film.