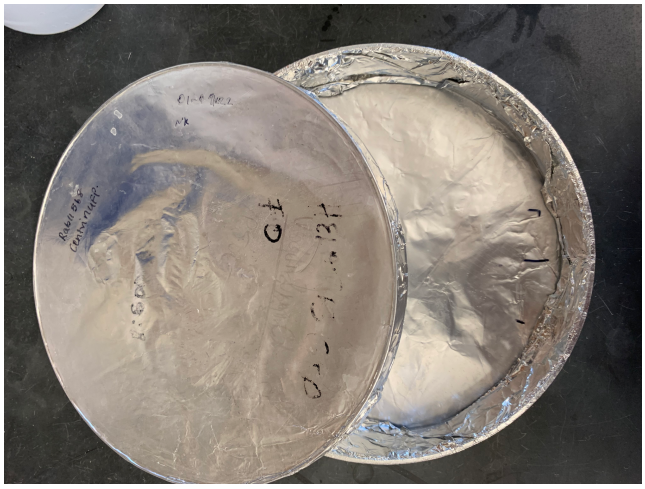
General Immunostaining Protocol:

(Good for membrane bound proteins)

1. Fix in 4% PFA at Room Temperature for 20 minutes
2. Wash in 1X PBS 3x's 5 min each
3. Quench in 50 mM NH4Cl (ammonium chloride; Fisher cat. A661-500) at Room Temperature for 10 min
4. Repeat step 2
5. 0.1% TX-100 for 4 min at Room Temperature (make a 20% filter sterilized stock)
6. Repeat step 2
7. Block in 2% Donkey serum (Jackson ImmunoResearch, cat. 017-000-121) or 2% other serum depending on the secondary used (dilute in 1X PBS) for 30 min at RT
8. Wash in 1X PBS for 5 min
9. Incubate in primary antibody at Room Temperature for 1 hour (dilution Buffer: 0.2% Donkey Serum, 0.1% Tween20 in PBS)
    a. Typically use 80-100 µl (can use as little as 30 to 50 depending on coverslip size) and place coverslip cell side up, on top of parafilm in a chamber (pictured on right) with a moist kimwipe to prevent coverslips from drying. Keep chamber covered.
    b. After incubation antibodies can be removed and stored to use again (depending on antibody).



10. Repeat step 2.
11. Incubate secondary antibody at Room Temperatute for 1 hour.
    a. Dilute secondaries 1:500-1:1000 depending on how secondary is stored (if it's diluted in glycerol, use 1:500). Use same dilution buffer as in 9.
12. Repeat step 2.
13. Gently Wash coverslips in water
14. Mount slides using vectashield (Vector Labs cat. H-1000-10) (~3ul) with nail polish or Prolong (Fisher Scientific cat. P36934) (~3ul). If using prolong let sit in the dark at room temp overnight or for 1 hour in 37°C.