General protocol: Fluorescent Immunohistochemistry (IHC):

Required Materials:

- ☐ Xylenes
  - Ensure xylenes are handled under sufficient ventilation (fume hood).
- ☐ 100% ethanol
- ☐ 95% ethanol
  - If more 95% ethanol is needed, dilute by adding 41.2mL distilled water to unopened 4L jug of 100% ethanol
- ☐ 70% EtOH
- ☐ Distilled water
- ☐ Antigen retrieval solution
  - Sodium citrate solution (IHCWorld):
    1. Add 2.94g trisodium citrate to 1000 mL distilled water (milliQ).
    2. Adjust pH to 6.0 or 9.0 (application dependent) using test strips by adding very small amounts of HCl (if too acidic, recover with very tiny amount of NaOH).
    3. Add 0.5mL Tween.
- ☐ Casein blocking buffer
- ☐ Tween 20
- ☐ Unconjugated primary antibodies
- ☐ Fluorophore-conjugated secondary antibodies
- ☐ Humidity chamber
- ☐ PBS (Stock or 10x)
- ☐ TrueBlack Lipofuscin Autofluorescence Quencher (TLAQ)(Cell Signaling Technology #92401)
  - Optional depending on the autofluorescence characteristics of your tissue of interest.
- ☐ DAPI
- ☐ Antifade aqueous mounting media (Invitrogen P36965)
  - ProLong™ Diamond Antifade Mountant
- ☐ Nail polish
  - Brighter colors are easier to see on glass.