General Slide staining Protocol

IMPORTANT\*\*\*\*\* It is important that you keep the tissue protected from light exposure as much as possible throughout this process!!!

1. Take slides from the freezer and thaw them for 30 min at RT
2. Encircle the tissue with “liquid blocker” or rubber cement and wait 20-30 minutes, or until the rubber cement is dry
3. Rinse slides 5x for 10 minutes in 0.05% PBS-T while on shaker
4. Treat for 60 min in blocking solution

* Making Blocking Solution:
  1. First we must make Dilution Buffer (DB) which can be prepared as follows
  2. Mix the following ingredients into 100ml deionized water:
     1. 1.74g of NaCl
     2. 0.3ml of Triton X-100
     3. 50mg of Bovine Serum Albumin (BSA)
     4. 10mg of Thimerisol
     5. 2.5ml of [Tris-HCl (1M, pH 7.2)](https://github.com/aspence/spencelab/wiki/Immunohistochemistry#Tris-HCl)
  3. Stir until dissolved. Store at 4C.
  4. Add the appropriate serum (10% of the total volume of your solution)

1. Take slides to the fridge and pipette the primary antibody onto the slides
   1. Preparing  Primary antibody
      1. Pipette the appropriate volume of Blocking Buffer into a vile
      2. Calculate the volume needed of each antibody and add to the blocking buffer
         1. DsRed 1:300
         2. Vglut1 1:400
         3. CTB 1:400
      3. Centrifuge to ensure the mixture is homogeneous
2. Allow slides to sit in the fridge overnight

Day Two

1. Remove the slides from the fridge and rinse slides 5x for 10 minutes in 1% PBS-T while on shaker
2. Apply secondary antibody
   1. Preparing Secondary antibody
      1. Pipette the appropriate volume of Dilution Buffer into a vile
      2. Calculate the vilume needed of each antibody and add to the Dilution Buffer
         1. Anti rabbit 594 1:400
         2. AF 488 1:400
         3. AF 647 1:200
      3. Centrifuge to ensure the mixture is homogeneous
3. Allow tissue to sit in the secondary 2 hours at room temperature
   1. You can also the tissue sit in the secondary in the fridge overnight
   2. If you are having issues with background, you may want to consider filtering your secondary, this can be done by extracting your solution with a syringe, screwing the appropriate size filter onto the end of the syringe, and filtering into a new vile
4. Rinse slides 5x for 10 minutes in 1% PBS-T while on shaker
5. Let the slides dry for 5-10 min
6. Remove the rubber cement from each slide
7. Apply approximately 200 ul of Fluromount-G per side (best to do one at a time)
8. Gently apply the coverslip and remove any excess liquid
9. Use clear nail polish around the edges to secure coverslip, and let dry in a dark place for 20-30 minutes