Immunocytochemsitry

- First day:
  1. Rinse cell coverslips two times with PBS(1X cold PBS wash, 75 rpm,5 min, RT)
  2. Fix cells with 3.7% formaldehye in PBS at room temperature, 75 rpm,10 min
  3. Rinse cell coverslips one time with PBS(1X cold PBS wash, 75 rpm, 5 min, RT)
  4. Permeabilize cells with 0.1 triton x-100 in PBS for 10 min, 75 rpm, RT
  5. Decant the solution and wash the cells in PBS, 5 min RT 75 rpm.
  6. Add blocking solution(1% BSA in PBS), 75 rpm, 30-60 min, RT
  7. Remove all the solution add 1st antibody(1:100, 40µl/coverslip), seal the dish with parafilm, with tissue inside, place in 4°C overnight

- Second day:
  1. Rinse cell coverslips three times with PBS(1X cold PBS wash, 75 rpm, 5 min, RT)
  2. Incubate cells with the secondary antibody (1:100, 40µl/coverslip) for 1 h at room temperature in the dark.
  3. Decant the secondary antibody solution and wash three times with PBS for 5 min each in the dark.
  4. Add DAPI (1:1000 dilution in blocking solution), 45 min RT in the dark.
  5. Decant the solution and wash the cells three times in PBS, 5 min each wash.
  6. Mount coverslip with a drop of mounting medium.
  7. Seal coverslip with nail polish to prevent drying and movement under microscope.
  8. Store in dark at -20°C