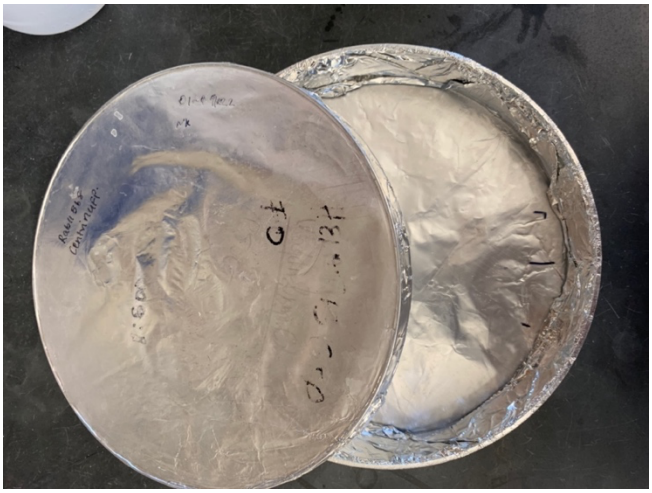
Heidi Hehnly Lab Protocol

Immunostaining Protocol for Cytoskeletal Proteins

This protocol is to be followed after fixing with either PFA or Methanol (see fixation protocol)

1. Align coverslips (#1.5, 12mm Round: Harvard Apparatus cat. 64-0712; 22mm Square: Harvard Apparatus cat. 64-0721) with cell coated surface facing up in a dark, humid chamber (foil wrapped 100cm cell culture plate (pictured on right) with parafilm covered bottoms and lined with KIMWIPES soaked in diH2O).



2. Block cells using PBST 1 for 1 hour at room temperature.
3. Incubate cells with primary antibodies (dilutions should be tested and can range from 1:50 to 1:10,000 depending on antibody) diluted in PBST 1, for 1 hour at room temperature (or overnight at 4°C).
4. Wash coverslips 10X with PBST 1.
5. Incubate cells with secondary (fluorescent) antibodies (Jackson Immuno Research Labs, Invitrogen , we use dilutions that range between 1:500 if antibodies diluted and stored in glycerol or 1:1000) diluted in PBST 1, for 1 hour at room temperature.
6. Wash cells 10X with PBST 1.

7. If immunostaining for Anti alpha tubulin that is directly conjugated to an Alexa Fluor (ex. AF555 to DM1A, EMD Milipore, Cat # 05-829-AF555), dilute antibody 1:200 to 1:500 depending on cell type in PBST 1 for 1 hour at room temperature (or 4°C overnight). If staining for actin, dilute 1 drop ActinRed v555 (Fisher Scientific, Cat # R37112) or Phalloidin 647 (Cell Signaling Technology cat. 8940S) in 1 mL PBS and incubate cells for 30 minutes (this is only done following a PFA fix). If staining for DNA, dilute 1 drop NucBlue or DAPI (Thermo Fisher Scientific cat. R37606 or Sigma-Aldrich cat. D9542-10MG) in 1 mL diH2O and incubate cells for 5 minutes.

8. Rinse cells with diH2O.
9. Mount on slides using either Prolong (Fisher Scientific cat. P36934) or Vectashield (Vector Labs cat. H-1000-10)

PBST 1 (Filter sterilize):

1X PBS

1% BSA (Bovine Serum Albumin; Fisher cat. BP1600-100)

0.5% Triton-X 100 (Fisher cat. BP151500)