Immunofluorescence staining:

Materials:
18X18 mm coverslips
Fixation buffer = 4% para-formaldehyde, 0.5% Triton X-100 in PBS (or in cytoskeleton stabilizing buffer)
Primary antibodies
Secondary antibodies
PBS
Wheaton glass jars (Columbia, Fisher #02-912-637)
Humidity chamber
Glass slide
Poly/aqua-mount solution
2% and 0.2% Bovine Serum Albumin (BSA) in PBS; made same day
Forceps X2
Large (1L) beakers

1. fix samples, variable time (minimum 45 minutes) at RT (use Wheaton jars); may store in fixation buffer at 4C overnight after RT.
2. Dip coverslips in two beakers of water and then place in 2% BSA solution
3. While coverslips in BSA, you may prepare antibody solutions. Dilute primary antibodies (usually 1:100) in 2.0 % BSA solution. Place 70 µl of the dilute antibody onto parafilm in humidity chamber and then place coverslip onto parafilm for incubation (incubate coverslips with primary antibody 37º C for 20-60 minutes).
4. After incubation with primary antibody, dip in two beakers of water and then place in 0.2 % BSA for 10 minutes X2 room temperature.
5. While coverslips in 0.2% BSA you may prepare secondary antibody dilution. Dilute secondary antibody (and phalloidin or DNA stain if applicable) in 2 % BSA solution (1:100) and then place diluted antibody solution onto parafilm. Place coverslips onto parafilm and incubate 37º C 20-60 minutes.
6. After secondary antibody incubation, dip in two beakers of water and then place coverslips into PBS for 10 min RT X2.
7. Dip in water and place coverslips onto one drop of poly/aqua mount on a glass slide. Very gently suction around perimeter of coverslip.
8. Store slides in square dish in refrigerator.