Immunofluoresence (24 well plate protocol)

Fixing and Permeablizing:

1. Make 4% PFA: Dissolve Paraformaldehyde in PBS and incubate at 70°C with occasional vortexing to dissolve
2. Aspirate off media from cells and wash with 500 μl 1xPBS
3. Aspirate off PBS and add 500 μl 4% PFA to fix cells. Incubate RT for 15 minutes.
4. Wash 2x with 500 μl 1x PBS
5. Aspirate off PBS and add 500 μl 0.1% Triton-X in PBS to permeablize cell membrane. Incubate RT for 10 minutes
6. Wash 2x with 500 μl 1x PBS (can store at 4°C or proceed)

Immunostaining:

7. Block 1 hour in 25 μl 1% BSA/PBS in humidity chamber (Petri plate, damp paper towel, parafilm)
8. Incubate in 25 μl 1° Ab (usually 1:200 in 1%BSA/PBS) for 1 hour in humidity chamber
9. Wash 2x with 500 μl 1x PBS
10. Incubate in 25 μl 2°Ab (1:200 in 1% BSA/PBS) for 40 minutes in humidity chamber. Place in dark so as to not bleach fluorescent 2° Ab
11. Wash 2x with 500 μl 1x PBS
12. Mount on slides with DAPI mounting media and seal with clear nail polish