Immunofluorescence

1. Wash cells 1X in 1X PBS
2. Fix cells in room temp (RT) 3% PFA for 15 min at RT. Discard any extra PFA, do not refreeze.
3. Wash cells 2X in 1X PBS
4. Permeabilize cells with fresh Blocking Solution for 20 min at 4ºC
5. Add primary antibodies (~2 ug/mL) in Blocking Solution (spin down diluted antibody for 5 min max speed) for 1 hr at RT (200 uL is sufficient to cover entire well of 4-well slide)
6. Wash cells 3X in 0.2% Triton X-100 in 1X PBS for 5 min each
7. Add secondary antibodies (Alexa dyes 1:500) in Blocking Solution (spin down mixture for 5 min max speed at RT) for 30 min (cover with foil to prevent light-induced bleaching from this point on)
8. Remove plastic chambers using black and white key apparatus
9. Wash cells 3X in 0.2% Triton X-100 in 1X PBS for 5 min each in coplin jars
10. Stain nuclei with DAPI (1 µg/mL; 5000X) in 1X PBS for 2 min in coplin jars
11. Wash cells once in 1X PBS in coplin jars
12. Add a small drop of mounting solution to each well and then add coverslip gently to prevent air bubbles
13. Air dry 15-30 min at RT (cover with foil to prevent light-induced bleaching)
14. Seal slides with nail polish
15. Store at 4ºC in the dark

Preparing 3% Paraformaldehyde (PFA)

3 g of PFA
10 mL 10X PBS (Mg and Ca free)
90 mL ddH_2O
Combine in 125 mL screw top bottle with stir bar

Heat at 90 ˚C while stirring on a stirplate in the hood with cap loosened to prevent bottle from exploding. It may take more than an hour to go into solution.

Cool to room temperature.
Add 10 uL 1M $MgCl_2$
Add 10 uL 1M $CaCl_2$

Filter with 0.45 um filter
Make 5 mL aliquots in 15 mL screw cap tubes
Store at -20 ˚C

Never refreeze thawed PFA.

Blocking Solution

1X PBS
3% BSA (kept at +4 C)
0.2% Triton X-100

Filter through 0.45 membrane.
Make fresh for each experiment.