Immunofluorescence

1. Wash cells 1X in 1X PBS
2. Fix cells in ice cold (-20 ˚C storage) 100% Methanol for 10 min in -20 ˚C.
3. Wash cells 3X in 1X PBS for 5 min each to rehydrate cells
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 0.1% Saponin 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 0.1% Saponin 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

Blocking Solution

1X PBS
3% BSA (kept at +4 C)
0.1% Saponin

Filter through 0.45 membrane.
Make fresh for each experiment.