# Immunofluorescence

Revised by J Sims
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## Procedure

1. Plate cells on coverslips to a density of 105 cells/well (2mL/well) in 6 well dishes.

2. Fix cells when they reach ~60-80% confluent.
   2.1. Make 4% paraformaldehyde/PBS- dissolve 4g paraformaldehyde in 100mL PBS, heat to 60°C for it to go into solution.
   2.2. Add 2mL paraformaldyhyde/well and incubate 10 min at RT.
   2.3. Aspirate paraformaldehyde and wash 3x10min with PBS

3. Permeabilize cells with 0.2% Triton X-100/PBS (200µL Triton/100mL PBS).

4. Incubate 5 min RT.

5. Aspirate Triton and wash 3x5min with PBS

6. Block cells in 5% donkey serum/PBS (5mL donkey serum/100mL PBS) for 1 hour at RT.

7. Aspirate seurm.

8. Add primary Ab, diluted in 5% donkey serum/PBS (125µL/coverslip) to the center of the coverslip. Try to move the coverslip to the center of the well to prevent the antibody from wicking off of the coverslip.

9. Incubate at 37°C for 1 hour in humidifier.
   (Or a Tupperware container lined with damp paper towels)

10. Add 2mL 5% donkey serum and wash 5 min (repeat 2 more times).

11. Aspirate serum and add secondary Ab, diluted in 5% donkey serum/PBS (125µL/coverslip) to the center of the coverslip.

12. Incubate at 37°C for 1 hour in humidifier.

13. Wash 3x10 min with 5% donkey serum/PBS.

14. Mount coverslips using mounting medium containing DAPI.
    14.1. Add one drop of mounting medium to slide.
    14.2. Rinse coverslip in dH2O.
    14.3. Place coverslip cells side down onto mounting medium.
    14.4. Blot off edges and let dry.

15. Store slides at 4°C