**Indirect immunofluorescence assay (IFA)**

**1. Fixation**

Fixative : 4% paraformaldehyde (PFA) in PBS for 10-12 minutes or

4% PFA/0.05% glutaldehyde (GA) in PBS for 10-12 minutes or

Choice of fixative depends on nature and localization of antigen intend to detect

Remove medium from IFAs, wash once with PBS and then fix

**2. Neutralization**

3-5 minutes with 0.1 M glycine/PBS

At this stage, either carry on with the next steps or store the IFA at 4°C

**3. Permeabilization and blocking**

Permeabilize cells with 0.2% Triton/PBS for 30 min on an orbital shaker

Block non-specific binding with 2% BSA/0.2% Triton/PBS for 30 min on an orbital shaker

**4. Incubation with primary antibody**

Incubate with primary antibody for 1 hour, RT, on an orbital shaker

Antibodies diluted in 2% BSA/0.2% Triton/PBS

Wash 3 times, each of 5 min with 0.2% Triton/PBS

**5. Incubation with secondary antibody**

Incubate with secondary antibody for 45 min at RT, in DARK, on an orbital shaker (the secondary antibodies are coupled to a fluorochrome, which is light sensitive)

Usually, commercial antibodies are diluted 1:3000 dilution in 2% BSA/0.2% Triton/PBS

Wash 3 times, each of 5 min with 0.2% Triton/PBS

**5. DAPI staining**

Wear gloves when handling DAPI!!!

1000x concentrated DAPI stock: 50 ug/mL in water, 0.5 mL aliquot, kept at -20°C.

Dilute DAPI stock (1000x concentrated) in PBS and stain cells for 5 min (overstaining with DAPI is not recommended)

Wash once with PBS

**6. Mounting**

Put around 10 uL drop of Fluoromount G on slide for each coverslip (try to avoid bubbles!!)

Take out coverslip with fine forceps, and mount it with cell layer facing down on slide

(Aliquots of Fluoromount G should be kept at 4°C)

**Storage**

Slides should be kept at RT/4°C at least 1 hour (for drying) before checking at the microscope

For longer term storage, slides should be kept dark at 4°C