# Immunofluorescence Protocol

### Setting up the cells

1. Autoclave coverslips and a pair of tweezers which you are going to use to place the coverslips into the culture dishes.
2. Place up to three coverslips in one tissue culture dish, always work sterile under the hood !!!.
3. Add 10 ml of culture media to each dish.
4. After splitting your cells, add appropriate amount of cells to each dish. Note: cells need some time to adhere on coverslips, therefore you should give the cells at least 24 hr. after the passage to do so and before you procede with the protocol. The number of cells added to each dish depends on the individual cell growth rate. However, at the time of fixation cells should be ~ 40-50% confluent. To be able to get good images under the microscope you need single isolated cells.

### For further processing:

1. Draw off culture media
2. Rinse the dish 2x with warm PBS.
3. Transfer coverslips to 6 well culture dishes/one coverslip per well. Note: It is easier for the further treatment of the coverslips , if you line the bottom of the wells with parafilm before hand.

### Fixation

1. Add 30ul of 4% Paraformaldehyde for 30 min @ room-temperature (RT).
2. Wash 1x with PBS for 5 min.

   **Note:** During washing period always place 6 well dishes on orbital shaker.

### Permeabilization

1. Add 30ul of 1% Triton in PBS for 10 min @ RT
2. Wash 1x with PBS/ 0.02% Tween 20 for 5 min
3. Wash 1x with PBS/0.02% Tween 20/ 1% BSA for 5 min

### Primary antibody

Prepare 1o-antibodies in PBS + 3% BSA, dilute antibodies according to the recommended manufacturer specification data sheet that is supplied with the antibody. If you normally use a polyclonl antibody in a 1:1000 dilution in a western blot, you can probably use it in a 1:30 dilution for immunofluorescence labelling.

1. Add 30ul of 1o antiboby in PBS/3% BSA

2. Incubate in a humidified chamber for 45 min @ 37oC. (We normally used a waterbath).

### Washes

1. Wash with PBS/0.02% Tween 20/1% BSA for 5 min

### Secondary antibody

Prepare 2o antibodies in PBS/3% BSA (dilute antibodies according to the recommended manufacturer specification data sheet)

1. Add 30ul of each antibody per well
2. Incubate in humidified chamber for 45 min @ 37oC

**Note:** If you have got more then two antibodies repeat antibody and wash steps as often as needed. After adding your last antibody proceed as following:

- **Washes**

1. Wash with PBS/ 0.02% Tween 20 for 5 min
2. Wash with PBS for 5 min

### Dry

1. Dry @ 37oC in oven (we normally use bacterial incubator) for ~ 45 min

### Mounting

Mounting media: mix PBS, 50-60% glycerol, 2.5% 1,4 diazobicyclo(2,2,2)-octane = Dabco Quencher

1. Use 10-15ul per coverslip.
2. Apply mountant on slide and add coverslip with cells facing mountant on top.
3. Seal coverslides with Nail Polish - don`t forget to label slides.