Indirect immunofluorescence labelling – Flow Cytometry
(Ideally $2x10^6$ cells are needed for each sample)

This protocol can be used for fixed cells as well as freshly isolated or lifted viable cells. It is recommended that when labelling live cells, antibodies be made up in PBS or PBS/BSA.

Date……..

Antibody……

(Cells plated on…….)

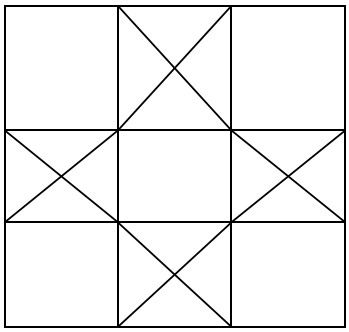
Tube 1 (IgG control) =……..
Tube 2 (Antibody 1) =…….
Tube 3 (……..) =…….
Tube 4 (……..) =…….
Tube 5 (……..) =…….

Blocking serum = (1:20) 500µl - 1ml needed for each sample
…….
…….
Primary antibody = (10µg/ml) 500µl needed for each sample
…….
…….
…….
…….
IgG control = (10µg/ml) 500µl needed for each sample
…….
…….
Secondary antibody = (10µg/ml) 500µl needed for each sample
…….

- Wash plated cells with sterile PBS.
- Add 1ml Accutase to each well of 6 well plate and incubate at 37°C for 5-10 minutes or until cells become rounded and begin to detach.
- Pipette cells into 15ml tube and wash wells with sterile PBS.
- Centrifuge cells at 20krpm for 5 minutes then aspirate media.

- Resuspend in sufficient volume of PBS and count cells.

Total volume = ……
Dilution = ……



Average = ……....
Total No. of cells = ……

- Pipette cell suspension into …… 15ml tubes and centrifuge at 20krpm for 5 minutes, then aspirate supernatant. **No. of cells per tube = ……...**
- Make up blocking serum.
- Add blocking serum to each tube, re-suspend and incubate for 15 minutes at 4°C.
  Ready at……..
- Make up primary antibody and IgG control.
- Separate cells into relevant tubes and centrifuge at 2000 rpm for 5 minutes
- Add primary antibody/IgG control to each tube, re-suspend and incubate for 30 minutes at 4°C.
  Ready at……..
- Wash in PBS and centrifuge at 2000 rpm for 5 minutes.
- Repeat above step.
- Make up secondary antibody.
- Add secondary antibody antibody to each tube, re-suspend and incubate for 30 minutes at 4˚C (wrapped in foil). **Ready at…….**
- Centrifuge cells at 20krpm for 5 minutes then aspirate supernatant, wash in 1ml PBS.
- Repeat above step.
- Aspirate and add a further 1ml PBS.
- Filter cells into FACS tubes.
- View on FACS machine.

Notes: