Direct 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.

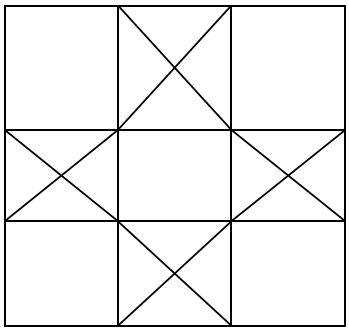
Date……..

Antibody……

Tube 1 – MIG RPE
Tube 2 – MIG FITC
Tube 3 – CD105 RPE
Tube 4 – CD166 FITC
Tube 5 – CD105 RPE/CD166 FITC

Primary antibody = 1:50 (10μg/ml), 500μl needed for each sample
IgG control = 1:50 (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. **Ready at…….**
- 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 = ……...**
- Add conjugated primary antibody or IgG control to each tube, re-suspend and incubate for 45 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: