

## Surface Staining –Fixed Samples

1. Harvest the cells
2. Spin 5 min at 4°C, 1500 rpm and discard the supernatant
3. Wash the cells with 10ml of staining buffer
4. Spin 5 min at 4°C, 1500 rpm and discard the supernatant
5. Resuspend the pellet in 1ml of staining buffer and count the cells using trypan blue to determine viability
6. Resuspend the cells in staining buffer at  $10 \times 10^6$ /ml
7. Distribute in eppendorf tubes 100µl of cell suspension equivalent to  $1 \times 10^6$  cells, add 900µl of PBS 1X w/o serum
1. Spin 5 min at 4°C, 1500 rpm
2. Remove the supernatant and resuspend the cell pellet in 100µl of LIVE/DEAD® Fixable Dead Cell Stain diluted (1/1000 PBS 1X)
3. Incubate at RT for 20 minutes protected from light with foil
4. Wash the cells with 1ml of staining buffer and spin the cell.
5. Perform surface staining if required
6. After incubation with the antibody mix, wash the cells with 1ml of staining buffer
8. Spin and resuspend the pellet in 100µl of PBS1X + 100µl of PFA 4%. Transfer the cell suspension to a facs tube suitable for acquisition in the cytometer. For the LSRFortessa use: Polystyrene Round-Bottom tubes, Ref. 352058 Falcon
9. Acquire the samples

### **Staining Buffer:** PBS 1X-2% FCS-2mM EDTA

1000 ml PBS 1X

Take out 24 ml and add:

20ml FCS

4ml EDTA 0.5M

### **Fixation solution:** PFA 4%

For 100ml of 4% paraformaldehyde, add 80 mL of 1X PBS to a glass beaker on a stir plate in a ventilated hood. Heat while stirring to approximately 60 °C. Take care that the solution does not boil.

Add 4g of paraformaldehyde powder to the heated PBS solution. The powder will not immediately dissolve into solution. Slowly raise the pH by adding 1N NaOH dropwise from a pipette until the solution clears.

Once the paraformaldehyde is dissolved, cool to room temperature and adjust pH to 7.2 with 0.1 M NaOH or 0.1 M HCl. Adjust the volume of the solution to 100ml with 1X PBS. The solution can be aliquoted and frozen or stored protected from light at 2-8 °C for up to one month (From R&D systems protocols webpage).