Cell surface marker staining (Phenotyping)

Direct staining

1. Harvest cells and wash them with PBS.
2. Incubate ~1x106 cells in 100µl containing the optimal amount of pre-titrated directly conjugated antibody. For multicolor experiments for several surface antigens the antibodies can be added simultaneously. Incubate protected from light 30-45 min at 4°C or 15-30 min at RT.
3. Wash cells with PBS or staining buffer, pellet cells and remove the supernatant.
4. If desired cells can be fixed at this point using e.g. 4% PFA for 15 min at RT. After fixation cells are pelleted and suspended in 500µl PBS or staining buffer and stored at 4°C until analyzed.
5. If fixation is not needed cells are suspended at point 3 in 500µl staining buffer or PBS and stored at 4°C until analyzed (do not store longer than 7 days).

The following controls are good to include:

1. Unstained cells to determine the baseline for the cells and distinguish the autofluorescence of the cells from real signal.
2. A stained sample that is known to be negative for the marker in use – as a negative staining control to reveal unspecific staining.
3. Stain the cells with an immunoglobulin isotype control of irrelevant specificity. The isotype control antibody should be used at the same concentration as the specific antibody.
4. When doing multicolor experiments electronic compensation to correct spectral overlap is usually needed. Comp beads or cells stained with individual antibodies can be used as compensation controls

Home made staining buffer

- PBS
- 2% FCS
- 0,09% sodium azide

adjust pH to 7,4 filter and store at 4°C