

Preparing Cells for Fluorescence-Activated Cell Sorting

- Prepare a high density cell suspension in the range between 5×10^6 and 10×10^6 cells/ml if possible.
- When using adherent cell lines, choose appropriate detaching procedure that does not interfere with surface markers used for sorting.
- Keep adherent cell lines or primary cells on ice prior to sorting.
- For cell preparation use PBS or HBSS without Ca^{2+} and Mg^{2+} supplemented with 2% BSA and 2mM EDTA.
- If necessary use DNase II at a concentration of 10U/ml to prevent clumping of fragile cells.
- When using antibodies for labelling cell sub-populations, please get in contact beforehand to ensure that our filter settings fit your selection of fluorochromes.
- Perform antibody labelling on ice and incubate in the dark.
- Directly prior sorting pass the cell suspension through a cell-strainer using 5ml Polystyrene round-bottom Tubes with a cell-strainer cap with $35\mu\text{m}$ nylon mesh (Falcon Ref-No. 352235).