Sample preparation for cytometer usage (Sorting and Analysis)

General

Single cell suspensions should be as clean as possible with minimal debris and free of clumps and aggregates.
Cell suspension media should contain both protein and EDTA. We recommend either **PBS/2% FBS/2-5 mM EDTA or PBS/0.5%/BSA 2-5 mM EDTA.** Do not use media containing phenol red (DMEM or RPMI). To minimize particles and contamination, the FACS buffer (or at least the FCS or BSA) should be filtered through a 0.22uM filter.
To remove debris and excess antibody, please wash samples at least 3 x in FACS buffer.
To remove aggregates and clumps, all samples should be filtered IMMEDIATELY prior to sorting or passing on an analyser (40υ cell strainer, Falcon cat. no. 352235 or mesh filter sheets). This should be done at the machine in either the sorting room or the FACS lab where the machines are located.

Special requirements for FACS (Cell sorting)

Ideal cell concentration for sorting lymphocytes is from 5 – 7 x 10^6/ml. Cells should be counted AFTER staining and washing to monitor both viability and cell concentration.
Ideal cell concentration for larger, more sticky cells (epithelial cells, tumour cells, cell lines etc…) is 3 x 10^6/ml. This allows the cells to be sorted without having to apply excessive extra pressure to the sample tube. Cell viability will be better at lower pressures.
Unstained cells and single stains of each fluorochrome used in the experiment (compensation controls) must be provided for each sort.
We **strongly recommend** a viability dye marker (DAPI, 7-AAD or a fixable live/dead marker such as Vivid Aqua) to be added in order to efficiently sort viable cells of interest.
Collection tubes should have the inner walls pre-coated with a protein containing solution to minimize sticking and cell loss (unless sorting into medium in plates or directly into nucleotide preparation solutions). Completely fill the tubes with either 100% FCS or FACS buffer containing FCS and let sit as long as possible. Empty the tube just prior to sorting and leave a small amount of FACS buffer in the bottom in which to collect the cells. Sample viability and recovery will be maximized.
For single cell sorting into plates (96 well, 384 well, etc…), the concentration of the sample should not exceed 1x10^6/ml to optimize efficiency and cell recovery.

Sample analysis guidelines

All samples regardless of their origin MUST be filtered IMMEDIATELY prior to passing on the analysers (see filter recommendations above).
Unstained cells and single stains of each fluorochrome used (compensation controls) should be provided for each experiment. The addition of a viability dye (DAPI, vivid or zombie dyes) to eliminate dead cells is strongly recommended.
Users should run all samples at the 'low' setting on all machines to minimize clogging and maintain fluidics integrity. The ideal maximum events/sec above the threshold is around 5000 events/sec for non-sticky cell populations. For large and/or sticky cells (BM, cultured cells, tumour cells, epithelial cells etc), slower flow rates are recommended.