SAMPLE PREPARATION GUIDELINE
FOR CELL SORTING IN IBG FLOW CORE FACILITY

SAMPLE TUBES

All samples must be loaded onto the sorter in 12x7.5ml BD FACS tubes.

TEMPERATURE

Keep your samples on ice, this will prevent formation of aggregates, unless your protocol will not allow this.

SINGLE CELLS

You MUST filter your samples just before running them on Sorter

SORTING BUFFER

For best results use PBS supplemented with 1% dialysed FBS. Using the lowest possible concentration of protein will reduce auto fluorescence.

If your cells have a tendency to clump use Ca/Mg ++ free PBS.
Add EDTA (1mM--5mM) to the buffer to prevent formation of aggregates.
Add 25--50ug/ml of DNAse I and 5mM magnesium chloride hexahydrate
if cells are clumping due to cell death.

LIVE /DEAD DISCRIMINATION

Always use a viability dye to exclude dead cells.
When staining cells with conventional dead cell exclusion dyes. For Aria III, **DAPI** is recommended, add just before running your stained sample. After 5 minutes incubation wash the viability stain off before running your sort sample.
There is a good range of new viability dyes available for many fluorescence channels, which are also fixable. e.g Live /dead aqua, eFluor 450, and eFluor780. Stain the cells with these viability dyes before surface staining.

FIXED SAMPLES

If your sort samples have been fixed for any reason, ALWAYS ensure the cells are washed twice to remove fixative before sorting.

SAMPLE CONCENTRATION

Too few cells and the sort will take longer than necessary. Too many cells can cause reduced purity and more chance of blockages.

Here are some guidelines:

If you have fewer then 5 x 10^5cells put them into a volume of 1 ml.

Sample concentration for sorting should be 1x 10^6cells/ml to 20x10^6cells/ml depending on the cell type.

First time, please bring sample as 5-10x10^6cells/ml.

COMPENSATION CONTROLS

Please provide all the necessary controls in order for your sort to be valid.

If have any question, please ask for advice from specialist.

Negative Control:

0.5 to 1x 10^6cells/ml non stained cells.

Single stained compensationcontrols:

Provide 0.5 to 1x10^6cells/ml of single stained cells for every colour you are using.

If you intend to use comp beads to set up the compensation bring:

Unstained Cells: 0.5 to 1x 10^6cells/ml.

Unstained beads Single stained beads for each color, using the same antibodies you will use to stain your cells.

FLUORESCENCE MINUS ONE GATING CONTROLS

If antigen expression is low, or differential within a population, then FMO controls can be used to set gates for positive cells. FMO control tubes are stained up with all the antibodies in your panel minus one.

CONTROLS FOR TRANSFECTED CELLS

Please bring mock-transfected cells (no FP expressed)

If your sample cells are going to express several fluorescent proteins simultaneously, please bring along the control cell lines for each fluorescent protein you will be using, which express a single Fluorescent protein.

COLLECTION MEDIA

Should be optimized for your cells:

Fill the collection tubes>1/3 with your cell culture media supplemented with 20-50%FBS depending how fussy your cells are.

CELL RECOVER

It is advisable to count your labeled cells just before sorting. You always get back less cells than you expect and than the machine counts. Cells are lost through washing steps, filtration, sort aborts, cells sticking to the collection tubes.

It would be a reasonable assumption that you will ultimately recover

50% of the cells you started out with.

How many cells must you start with?

This is going to depend on the incidence of the cells of interest in your total population.

See the table below as a rough guide. The data given is based on sorting $1x10^6$ cells of the target population.

Frequency of cells of interest	10%	1.0%	0.1%
Starting number of cells required	$1x10^7$	$1x10^8$	$1x10^9$
Time to sort $1x10^6$	30(minutes)	300 (5 hours)	3000 (50 hours)