

SAMPLE PREPARATION FOR FACS SORTING

**Whatever your cell type, samples must be of a good quality, high viability single cell suspension. Cells with low cell viability will not respond well to the stress of sorting, and the presence of dead cells compromises sort efficiency.**

**STERILITY**

 Do you require a sterile sort from us? The machine setup time for this is two hours. You need to ensure you prepare your sort samples in sterile conditions and ensure that that all FACS buffer/ collection media/ antibodies/tubes/nitex/pipette tips you use are sterile!

**SAMPLE TUBES**

All samples must be loaded onto the analysers/sorters in 12x7.5ml BD FACS tubes. These are available from MVLS Stores.

 TUBE POLY. 6ML - pk/125 £9.01 Code: TU.P06/1-

 TUBE POLY .6ML CAPPED £14.01 Code: TU.P06/2-

**TEMPERATURE**

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

**SINGLE CELLS**

You must filter your samples just before running them through the machine – use:

Nitex nylon mesh OR

or BD falcon cell strainers 40-70 micron)

or BD FACS tubes with cell strainer cap ref 352235)

or Cell Trics, which are available from Partec UK.

**FACS BUFFER – basic recipe**

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

The high pressure during sorting can cause the sort buffer to become basic. Add HEPES buffer (final conc 25mM) to maintain the pH at 7.0 - 8.0

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 10U/ml of DNAse II 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, e.g PI, 7-AAD, Hoechst, DAPI, add just before running your stained sample. After 5 minutes incubation wash the viability stain off before running your sort sample. NB these stains cannot be fixed)

 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. Care must be taken to use the appropriate buffer. Follow the instruction on the data sheet.

**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 compromising viability. Too many cells can cause reduced purity and more chance of blockages.

 Here are some guidelines:

* If you have fewer than 5 x 105 cells put them into a volume of 1 ml.
* Sample concentration for sorting should be 1 x 106 cells/ml to 2x107 cells/ml depending on the cell type.
* Low speed sorting (<=5000events/sec) bring cells at 3x106 cells /ml
* High speed sorting (<20,000 events/sec) bring cells at 1-2 x107/ml

**COMPENSATION CONTROLS**

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

 Negative Control: 0.5 to 1x 106 cells/ml non-stained cells.

Single stained compensation controls: Provide 0.5 to 1x 105 cells/ml of single stained cells for every colour you are using. Include negative cells in each single colour control tube.

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

* Unstained Cells: 0.5 to 1x 106 cells/ml.
* Unstained beads
* Single stained beads for each colour, 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 only a single Fluorescent protein.

**COLLECTION TUBES**

 Please provide collection tubes for the purified post sort) cells. 2 way sorting: Cells are sorted into 15mL Falcon tubes. 4 way sorting: Cells are sorted into 12x75mm FACS tubes

 ***Polypropylene tubes are better than polystyrene as cells adhere less.***

In order to prevent cells sticking to the sides of the tubes, you can pre coat the tubes, filling them with the serum for 30 minutes before the sort, or you overnight at 4ºC with 10% BSA. We can also sort into multi well plates; and onto microscope slides.

**COLLECTION MEDIA**

 Should be optimized for your cells:

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

DNA: PCR mix or lysis buffer

RNA : Trizol, TRizol LS, RLT buffer.

 Please bring DEPC water the day before your sort .

 **CELL RECOVERY**

 What you put in is not what you get out! It is advisable to count your labelled 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 1x106 cells of the target population.

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**SORT TIME**

 Cell sorting can be very slow depending on the type of cell to be sorted and the purity desired. Generally speaking the bigger the cell the slower the sort. Adherent and fragile cells will be sorted more slowly. Low frequency populations are sorted at just a few events per second. It will take 1 hour to set up the sorter, 2 hours for a sterile sort. 15 minutes to run the compensation and FMO tubes and set the sort gates, then after the sort is finished, up to 10 minutes for post sort analysis.