"How to bring your cells for sorting"

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Cell sorting, like any flow cytometry experiment, requires proper controls to set it up correctly and ensure you are separating the right cell population. When you are planning to sort:

- Make sure you have an **unstained control tube** of your cells.
- In case of multi-color staining, you will need **single stained tubes** for each one of the fluorochromes (for spectral overlap compensation in multicolor assays).
- In instance where the position of gates is not definite, we will recommend you to include **FMO** (Fluorescence minus One) control tubes.
- We highly recommend a **dead cell exclusion dye** for any sort that ultimately increases the yield of the sort. In such case, you will need to bring a separate viability control of dead cells; a simple solution is to heat kill your cells at 65^0C for 10 min and mix with live cells. Many dyes are available of which some are available in our facility and are ready to use. Contact us for best practice with respect to your experimental design.

For optimal cell recovery, we highly recommend that you bring your samples in an ice bucket to minimize cellular activity while waiting to be sorted and post sorting. Please remember that while being sorted, both the cell sample tube and the collection tubes are kept at 4^0C, by default.
Make sure to indicate us if your cells are sensitive to temp.

When sorting directly into plates, room temperature is recommended.

If this is your first time sorting, it is best to contact us prior your sort appointment.

Sample Tube Medium:

Suspend the cells in: 1xPBS (**Ca/Mg++ free**) + 2% serum (e.g. BSA/ FCS/ FBS), double amount of antibiotics (usually 2%). Please note that calcium and magnesium free media is vital to maintain cells at a single cell suspension. Sample tubes to be use with Aria sorter are 15ml conical tubes or 5ml polystyrene FACS tubes while with Melody sorter you may use only 5ml tubes.

If you are planning a long sort, the addition of 10mM HEPES will guarantee a stable pH throughout the sort and is recommended.

For cell lines or adherent cells: the addition of 1-5mM EDTA is recommended to avoid cell aggregation. The correct amount of EDTA should be empirically determined as some cells are sensitive to high EDTA concentration.

For samples with high percentage of dead cells: The addition of 100ug/mL DNase to the Sample medium will help reduce DNA associated clumps.

Media containing Phenol Red is not recommended as it will increase background fluorescence and hamper sensitivity.

Make sure to FILTER your cells using 40-micron cell strainers right before you start sorting.

In case needed, falcon tubes with strainer caps are available for purchasing at the BCF.

Cell Concentration:

Cells concentration depends greatly on size, type and fragility of your cells, which in turns dictate the nozzle size to be used while sorting.

In FACS Aria nozzles are available in sizes of 70, 85, 100 and 130µm while in FACS Melody only the 100µm nozzle is available.

Adjust concentration according to the following table:

Cell Type	Nozzle size	Concentration
Lymphocytes or small cells	70 um	7.5 -12 x 10^6 cells/ml
Activated lymphocytes, cell lines or cells <15 um in diameter	85-100 um	5 - 7.5 x 10^6 cells/ml
Fragile cells, cell lines or cells >15 um in diameter	130 um	3 - 5 x 10^6 cells/ml

In case of small amount of cells – suspend in, at least, 0.3 ml Sample buffer.

Generally, the size of the cell being sorted must be about 1/5 the diameter of the nozzle. If you have a cell line – choose a bigger nozzle e.g. 100 or 130. When working with primary cells you may choose a smaller one.

Prior sorting, you should have an idea of how many sub-populations you have and which one/s you'd like to collect, in addition to the expected percentage of the sub-populations you are interested in from the whole sample. For instance, if the subset you want to sort is 5% of the total cells and you need 1 Million cells for your downstream experiment, then in theory you would need to run 20 Million cells through the sorter. However, depending on the quality of your sample and “aborted” events, the actual yield is typically 50-90%. Therefore it is recommended that you bring, at least, twice as more cells to the sorter than you would need.

Collection Tube and media:

The AriaIII sorter is designed to sort into 5ml FACS tubes, 15ml conical tubes and Eppendorf tubes, as well as to a variety of plates ranging from 6 to 384 wells and onto slides or into petri dishes. Note that when sorting 4 populations simultaneously only the 5ml FACS tubes or Eppendorf tubes can be used. Sorting of 2 populations simultaneously can also be done into the 15ml conical tubes. For rare populations, it is best to sort directly into 1.5ml Eppendorf tubes.

The Melody sorter is designed to sort 2 populations simultaneously into 5 ml FACS tubes, Eppendorf tubes and plates ranging from 6 to 384 wells.

Note, that polypropylene FACS tubes are a better choice over polystyrene as they build up less static charge during the sort resulting in a higher post-sort yield.

Prepare collection tubes that contain growth media+ 20% serum (FBS/FCS), double amount of antibiotic (usually 2%) and any other goodies your cells of interest require.

Amount of medium in the collection tube:

- Sorting into 15 ml tube: 5ml of collection media
- Sorting into 5 ml tube: 1ml of collection media
- Sorting into 96 wells plate: 200µl collection media per well

It is recommended to bring extra amount of media and additional collection tubes.

Make sure to spin down and wash your cells post sort and resuspend in appropriate media. We can determine the purity of the sort by taking a small volume post-sort and re-run it on the sorter. We also recommend you to perform a post-sort cell count with trypan blue to estimate the quality of the sort preformed.

Please contact us in advance to communicate your requirements so we can advise you on the best choices for your experiment.

Estimated sorting time:

Sorting time can vary greatly due to sort conditions.

For the typical low-pressure sort with the 100 µm nozzle, you can estimate that we'll run a threshold rate of about 5000 cells/second. This translates to about 20 million cells per hour. For the typical high pressure sort with the 70 µm nozzle, you can estimate about 10,000 cells/second, translating to around 36 million cells per hour.

The table below illustrates some of the sort times required for the collection of cells from different starting populations.

Time needed to sort assuming a rate of 5,000 cells/sec and 80% recovery rate	
Required No. of cells	Desired cells as percent of total cells:

	0.1%	1%	5%	10%	50%
1000	~4 mins	~0.5 min	~6 Secs	A sec	A sec
10,000	~42 mins	~4 mins	~1 min	~1 min	A few secs
100,000	~7 Hours	~42 mins	~8 mins	~4 mins	A min
1,000,000	~70 Hours	~7 Hours	~1.4 Hours	~42 mins	~8 mins
10,000,000	~11.5 Days	~ 1 Day	~14 Hours	~7 Hours	~1.4 Hours

Note that the minimum reservation time for the Aria sorter is 3 hours; this includes a mandatory 30 min post-sort cleaning procedure. If you do not need the remaining 2.5 hours to sort, please let us know in advance and we will update your reservation accordingly as there is a charge for an order that has not been used up.