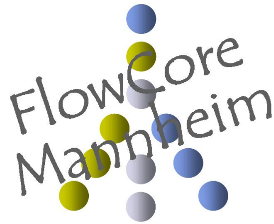

FAQ Cell Sorting

FlowCore Mannheim: Cell Sorting Core Facility

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(1) What do I have to do to have the cells sorted?
Simply contact us by mail or phone. If you plan a basic sorting experiment (e.g. GFP) a first contact by phone is sufficient. However, sometimes a personal meeting before the first sorting is advisable so that all requirements can be discussed.

(2) How to arrange an appointment? (see assignment form)
The following information is important in order to calculate a reasonable time frame for the sorting.

- type of cell
- number of cells (when sorting the complete material is requested)
- type of fluorochromes used
- population definition and frequency

(3) What do I need for the sorting?

- Cells in single cells suspension, eventually filtered, that are to be sorted
- necessary controls
- collection tubes (15ml tubes, FACS tubes or 1,5ml tube) containing collection media (e.g. PBS, cell culture media)

- and, for first time sorting, a little more time than usual.

(4) How many cells do I need for the sorting procedure?

This question is most frequently asked. In order to answer this, the following information is required:

1. How *big* is the population to be sorted (~ percentage)?
2. How many cells do you require post sorting?
3. What is more important: High purity or total yield?
4. How fragile or how large are the cells?

The duration of the sorting procedure depends on all these parameters. Here is an example:
A subpopulation of 20% has to be sorted and 2 million cells are wanted as the final yield for the following experiment. In theory, you would need 10 million cells to run the sorting process (20% of 10 millions = 2 millions). However, the actual yield is usually around 75-95% of this theoretical value. The reason for this lies in the abort rate arising out of sorting problems. Please beware that the quality of the cells to be sorted has the largest influence to the actual yield. In order to receive good sorting results, such as purity and yield, the cells must be clump-free and consist of a single cell suspension. Therefore, we recommend using **25-50% more cells than required by the theoretical value** for the sorting procedure.

(5) How long does the sorting procedure take?

The duration of the sorting procedure depends on several parameters, for instance on the target yield (yield vs. purity), on the cell characteristics (fragile and large cells need lower pressure and speed using a larger nozzle), on the concentration of the cells (the time for sorting procedure depends on the dilution the cells; the more diluted the cells are, the longer it takes) and, of course, on the quality of the cells.

Examples of maximum event rates:

- *Primary lymphocytes with 70 µm nozzle and 70psi: 20.000/sec*
- *Primary lymphocytes with 85 µm nozzle and 45psi: 10.000/sec*
- *Primary lymphocytes with 100 µm nozzle and 20psi: 6000/sec*

For cell lines, the event rate depends on the size of the cells. You need about 30 minutes to sort around 18 million cells at a speed of 10.000/sec. In this example, we have a theoretical yield of 3.6 million cells (18 millions x 20% = 3.6 millions). But the actual yield is significantly lower if the cells are of poor quality (low vitality, clumping, etc.) or if you demand high purity.

Available nozzle size	Pressure	max. cell size	examples
70µm	70psi	~14µm	Lymphocytes, splenocytes
85µm	45psi	~17µm	Activated T cells, Jurkat
100µm	20psi	~20µm	HeLa, MCF-7, CHO

(6) What needs to be considered when preparing the cells?
The cells **have to** be filtered, ideally directly before the sorting procedure using a 30 µm or 70 µm cell strainer. The cell strainers can be provided by the FlowCore.

(7) How do the cells have to be resuspended for the sorting procedure?
The cells are resuspended at a concentration of 20-30 million (primary cells) or 5-10 million (cell lines) per millilitre in buffer. If you are uncertain, we recommend bringing the cells in a concentration that is rather too high than too low since they can always be diluted. An optimal cell preparation increases not only the vitality but also leads to a better yield.

(8) Which buffer do I have to use for the sorting procedure?
Buffers such as *calcium and magnesium free PBS* or *HBSS* can be used (→**best buffer for your cells!**). The addition of *10 mM Hepes pH 7.2* is recommended because it buffers the shifting of the pH value caused by the high pressure during the sorting procedure. In addition, many cells benefit from proteins in the buffer for example *2% FCS or BSA*. Please bear in mind that a serum concentration higher than 5% can lead to cell aggregation and clogging of the machine. For cell lines and adherent cells, the addition of *0.5 mM EDTA* is recommended. The concentration of EDTA can be raised up to 5mM for extremely sticky cells. In cell suspensions

with a high rate of dead cells, released DNA can bind to the cells and lead to serious clumping of the cells. Here, an additive of *10U/mL DNAse II* added to the sorting buffer may help.

(9) Which tubes do I need for sorting of the cells?

Sterile 15 mL conical tubes or sterile 5 mL FACS tubes are required. Sterile FACS tubes are provided by the Cell Sorting Core Facility.

(10) How do I have to transport the cells?

We recommend to transport the cells on ice and protected from light.

(11) What kind of controls do I have to bring along?

The following controls are recommended for flow cytometry experiments:

- Unstained cells as negative control
- Unstimulated/untreated cells as biological control
- Single stained cells or beads as compensation controls
- FMO (**f**luorescence **m**inus **o**ne) and vitality as gating control

(12) What kind of tubes are used for the collection of the cells?

In order to prevent high cell loss, the cells should be collected in polypropylene tubes or coated tubes (e.g. BSA or FCS; this prevents an accumulation of cells on the plastic wall). Add 3 mL buffer or medium to the tube since cells must never be sorted in an empty tube. For large cell numbers, 15 mL conical tubes are recommended, however, then only two populations can be sorted simultaneously. Add 1ml buffer when 5 mL FACS tubes are used or add 250µl buffer when 1,5ml tubes are used, four populations can be sorted at the same time. In addition, small cell numbers can be sorted directly into microtiter plates (6- to 96-well plates).

(13) Which fluorochromes can be measured?

It has to pre-checked that all fluorescent proteins can be measured (see performance features under equipment and online spectrum viewer tools). Not all fluorochromes displayed by microscope can also be measured at the flow cytometer! This has to be checked beforehand.

(14) What other preparations are required?
Before the cells can be sorted, they must be characterized (by fluorescence-microscope or by flow cytometry) beforehand. Please bring a recently dated print out with you the first time you come.
The completed request form has to be brought along as well and must have been filled in shortly beforehand. Please **come in time**!

(15) Is the sorting procedure under sterile conditions?
The sorting is accomplished under the sterile conditions the system allows (so called aseptically). The sterility of the sorter is controlled at regular intervals. The addition of antibiotics to the medium, however, is recommended for the sorted cells.