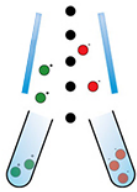


CELL SORTING GUIDE

Cell Sorting



BOOKING A SORTING SLOT AT THE FCCF



SAMPLE PREPARATION



IMPROVING THE QUALITY OF YOUR SORTS

Flow Cytometry is an extremely powerful technology that allows the individual measurement of physical and chemical characteristics of particles as they pass one by one through a light source. A major application of Flow Cytometry is the physical separation of cells and particles of interest from a heterogeneous population, this process is called cell sorting.

Booking a Sorting Slot at the FCCF

In order to get a slot for sorting please verify the availability of the sorters in the [ppms bookings system](#). In the REQUEST tab select book, fill up the sorting request format and submit.

Please take into account:

- The information that you provide in the request format allow us to determine the appropriate configuration of the sorter (regarding; nozzle size, pressure, lasers and optical components) in order to fits your requirements and get the best resolution out of your samples. For this reason, we ask you to be as precise as possible when filling the format and to avoid modifications of fluorochromes/fluorescent proteins the day of the sorting without telling us in advance.
- **Sorts are scheduled on a first-booked-first-served basis.** We will attempt to give priority to sorts that are time dependent e.g. on species availability (flies, plants) and we do expect a reciprocal degree of flexibility from our users. If your sort has to be moved or cancelled the facility will let you know as soon as this is noticed and proceed to re-schedule it to the nearest available time.
- Let us know if problems occurred subsequent to your sort. This could be low viability, poor cell numbers, contamination or some other problem. If we don't know about them, we can't do anything about it and we could potentially affect other people's sorts. Please take the time to provide feedback on your sorts.

Sample Preparation

Successful sorting depends almost entirely on the quality of the input sample. Make sure your samples fulfil the following specifications:

1. Bring your cell suspension exclusively in FACS Buffer containing: PBS 1X + 2% BSA + 2,5 mM EDTA. EDTA stock solution at 0.5M pH8.0 is usually available at EMBL kitchen. Please do not replace BSA by Foetal Calf Serum (FCS).
2. Filter your cells with a cell strainer of 40 µm pore size and transfer the sample to the appropriate tube: BD Falcon polypropylene round-bottom tubes, ref. 352063. If you do not have the appropriate material, come to the facility and we will provide tubes + cell strainers while you order them.
3. The sample should have a density of approximately 5-10 million/mL. If you have less than 5×10^6 cells put them into the minimal volume of 500 µL.

Bring to the sorting

- Negative controls and individual positive controls of your staining in order to set sorting gates appropriately.
- Extra 5mL of FACS Buffer in case further dilution of your sample is required.
- Collection tubes or plates with **fresh collection medium + antibiotics**.

Additional Tips:

- Make all cell preparations strictly on ice, unless otherwise stated in your protocol. Keeping the sample cool could in some cases improve the viability of the cells.
- **Collect your sorted cells in fresh complete medium with antibiotics**, especially if the cells will be culture after sort, to avoid risk of contamination.
- Dead cells can often be excluded by their light scatter characteristics. However this isn't always the case so we advise adding a **DNA-binding dye** that can be used as a marker to exclude dead cells from analysis and sort. The choice of dead cell exclusion dye depends on the colour combination of fluorochromes within the sample and the optical configuration of the sorter. Members of the facility could advise you on the appropriate DNA dye regarding your sample and sorter configuration.
- Dead cells can increase the incident of doublets and clumps due to release of DNA, which causes stickiness; Incubating the cells in the presence of DNase I (100ug/mL with 5mM MgCl₂) will help to reduce aggregates.