Cell Sorting at UNIFR

Access and Booking

- Cell sorting is a time-consuming technique that requires careful planning. Since the operator will perform the sorting, you need to pre-book your experiment **at least one week in advance.**
- Please be aware that some users need to book the sorters for a whole day. You are therefore advised to plan and book your experiments well in advance and to discuss your experimental protocol with us to ensure that sorting is doable.
- For booking, please first check the cell sorter availability on the Open IRIS calendar and send the completed sorting form by e-mail. We will confirm your booking, usually within 48h, and we will evaluate the time you need at the instrument. If it is your first booking, we will briefly meet to discuss your experiment protocol. There is a minimum of 1 hour booking slot for sorting. Don't hesitate to contact us when planning your experiments, especially if you do it for the first time!

Sample preparation

- Your cells should be prepared under the best possible conditions to preserve viability (gentle dissociation, suitable medium, 4°C). The happier the cells are, the better yields you will get.
- Samples should be single-cell suspensions of about 10-20 million per ml in Ca/Mg++ free HBSS or PBS buffer supplemented with 1-3% protein (either BSA or FCS) and 1-3 mM EDTA to minimize clustering.
- The cell concentration of samples for a plate sort (single cell cloning) should not exceed 1.5 million cells/ml and should not go below 0.5 million cells/ml.
- Count cells right before sorting, not before staining procedure, which often results in considerable cell loss. Always start staining with more cells than needed for the sort (e.g., twice the number).
- Please re-suspend your cells carefully before coming to the sort and filter them through a 40 micron cell strainer on a 5ml FACS tube just before coming to the sort.
- Your cell samples should be kept on ice and protected from light.
- Be aware that rare population will require a longer sorting time just because of the frequency of the detected events (for example if you want 100'000 cells that are 0.1% of your sample, it will take 1 hour).
- For rare populations, whenever possible, please pre-enrich them using MACS columns to reduce sorting times and recover cells in better shape.

- To preserve cell size and morphology after fixation protocols, put the cells back into PBS/FCS or similar, for running on the machines.
- We strongly recommend using viability dyes to exclude dead cells.

Things to bring with you for a sort

- Your samples for sorting
- **A sample of unstained cells (mandatory for the instrument set-up)**
- Single colour controls (mandatory for compensation, if more than 1 colour) on beads or on cells
- Extra-buffer to dilute your sample if needed
- Sterile medium to collect your sorted cells, around 1-2 ml per tube
- USB key to transfer the data