CELL SAMPLE PREPARATION GUIDE for CYTOMETRY LAB

Considerations before cell separation

Before bringing your samples for sorting, a series of parameters must be defined that will allow the proper selection of specific conditions for the sort. We need to know:

- Risk biohazard level. It defines the instrument we can use and the biosafety precautions.
- Type & size of the cells. It defines nozzle size & pressure.
- Markers & fluorochromes: it defines lasers, optic filters, controls required, etc.
- Relative abundacy of the target population and required purity. It defines the sort precision.
- Upstream application after sort: it defines external conditions to the instrument: sterility, refrigeration, etc.

Please, be sure that you put that information in your booking.

What do I need to bring to my sorting appointment?

- Samples pre-filtered through a 35 µm cell strainer if using the 70 µm nozzle for the sort (50 µm for the 100 µm nozzle); we have some in the Cytometry lab if needed.
- Cells in a single cell suspension in PBS or media containing less than 2% FCS. We normally call it FACS buffer and it has variable composition depending on cell type and experiment.
- Controls
- Vessel(s) to collect the cells of interest with buffer (culture medium/PBS etc. No volatile and harmful substances like 2-Mercaptoethanol or Trizol!). We recommend coating the inside walls of your collection vessels with your buffer of choice.

Which controls do I need?

- Unlabelled cells are used as a negative control
- Single stained cells or beads for compensation should contain both a negative and positive
- FMO controls in addition to experimental controls for accurate gate placement
- If your experimental markers will stay exactly the same eg. fluorochrome type and intensity for subsequent sorts, it is necessary only to bring the controls for the first sort booking.
- If using compensation beads instead of cells ensure they have the equivalent fluorescence.

What can I sort into?

- Eppendorf tubes 2mL (can contain around 300,000 smaller cells) or 1.5mL
- FACS tubes 5mL (each one can collect 4 million cells without media using 70 µm nozzle)
- Falcon tubes 15ml (2 populations simultaneously)
- Plates (6/12/24/96/384 wells).
- Slides or 6cm, 10cm plates.

How many cells should I bring?

The number of cells to be prepared for flow cytometric experiments can vary greatly and depends on the purpose of the flow cytometric experiment. Talk to us first if in doubt.

- 10 – 20 million cells can be sorted in 1 hour (depends on sample type & target population)
- For cell analysis, it is always a good start to start with 0.5 – 1.0 x 10^6 cells.
- For rare events is only possible if large sample numbers are acquired (plan booking time accordingly). FMO controls are useful here to draw a gate with confidence for the target population.

What density should the cells be at?

- For a 'bulk' sort into tubes around 10-20 million/ml
- For sorting into 96 well plates 1 – 2 million/ml
- Please bring additional buffer to dilute the samples if needed.
- Note, as a rule of thumb, approx. 1 hour sorting time is required for 1 ml sample volume
- If your sample contains only a few thousand cells, please keep the sample volume between 200 – 500 µL.

How many populations can I collect at once?

- 2 x 15 mL Falcon tubes
- 4 x Fusion or 6 x Astrios 5 mL FACS or eppendorf tubes at one time.
- Plate sorts are only one population per well at a time for any of the plate configurations

How much time should I reserve?

- To set up gates, voltages and record compensation = 15 – 30 minutes
- To sort around 10 million cells of the SAMPLE = 1 hour
- Instrument CLEANUP = 15 min

Why should I stain dead cells?

- Improves the quality of the sorted sample → more viable cells for upstream experiments.
- Dead cells tend to bind antibodies non-specifically, so you may analyze/sort false positives.
- Dead cells may lose expression of a previously expressed fluorescent protein, allowing false-negative cells to be sorted.
- The viability markers are usually DNA intercalants that DO NOT enter the DNA of intact cells.

What to do when your cells form aggregates?

We generally recommend to have a final concentration of 2-5 mM EDTA in your sample buffer. If your cells have a more pronounced tendency to form aggregates, you may consider <u>one</u> of the following modifications:

- Use calcium/magnesium free buffer for your cells
- Add EDTA (2 - 5 mM)

- Add 25 µg/ml DNAse I + 5 mM $MgCl_2$ (no EDTA then).
- Add 1% Accutase in sorting buffer

Is the sort sterile?

- No. However, the sheath in our sorters is filtered through a 0.2 µm filter and the sample lines are regularly cleaned with BD FACS Clean (bleach), detergent and distilled water, while surfaces are regularly liberally wiped with 70% ethanol. The sorters are technically classed as clean and not sterile. Some sorters have a Class II Safety Cabinet, which is designed for the operator biosafety (prevents air flow outwards); anyway, we can switch it on for your samples if you wish.
- If you want to culture your cells after sorting, we advise you to add pen/strep to your media. We regularly check for possible contamination by incubating samples from the cell sorters sorting stream sheath in culture media. We have never had any problems with contamination.

What can I do if I have low efficiency on my single-cell sorting?

- To sort one cell into a well and wait for it to grow is difficult to accomplish. We recommend to increase the %FBS in your culture media (10-20)%
- Additionaly, you can make a “conditioned culture media”, which is 50% fresh completed culture media and 50% 0,22µm pre filtered culture media from your plates in culture. It gives the cells a better chance to flourish as we are adding some grown factors to the collection media.

Samples for Biosafety level 2 Organisms (mostly human samples).

- Can be booked for sorting only in the Cat 2 room on the BD Fusion
- Samples must be bought to the lab capped and inside a sealed container eg. plastic box or polystyrene with a lid.
- Samples MUST be filtered before coming to the lab
- Cell sorting will be carried out on a BDFusion instrument in a Class II safety cabinet