General Cell Sorting Guidelines

Plan your experiment well and choose your fluorescent markers in agreement with the optical configuration of our sorter (See Optical Configuration of BD FACAria II).

Our cell sorter is equipped with Aerosol management system. However, we cannot analyze the samples that contain radioactivity and potentially infectious agents need to be discussed in advance with operators.

If you are using fluorescent antibodies, **stain cells according to your protocol**. Unstained / non-transfected cells are usually very helpful in estimating background signal. Further, consider the use of Fluorescent Minus One (FMO) controls and compensation samples depending on your panel requirements.

Sample preparation

- Your **cells** must be **viable**, and must be in a **single cell suspension**.
- For adherent cells, it is good idea to use the mildest detachment/dissociation method as possible. **Harshness Scale:** Trypsin > Collagenase A > Dispase > Liberase > Collagenase D > Accutase
- Resist the urge to save time and centrifuge your cells at high speed. You should limit your RCF to 100-200. This means 800 -1000 RPM on a standard benchtop centrifuge, spinning the sample 5-10 min.
- A maximum of 2 % FBS/BSA is recommended in the suspension medium of cells to be sorted because otherwise cells become sticky and the optical property of the stream start to change significantly. Try to avoid phenol-red in case you want to bring your cells in cell culture medium.

Basic sorting buffer:

- Phosphate Buffered Saline (PBS) or Hanks Balanced Salt Solution (HBSS) (Ca2+/ Mg2+ Free)
- 1 mM EDTA
- 25 mM HEPES, pH 7
- 1% FBS (Heat inactivated) or 1% Albumin
- Filter sterilize using a 0.2 µm filter. Store at 4 degrees.

Cell Type Specific Buffer Modifications:

1. **Clean Lymphocyte Populations:**

   The buffer can be simplified to just HBSS with 1% HBS.

2. **Sticky Cells:**

Raise the concentration of EDTA to 5 mM and use 1% BSA instead of FBS. EDTA helps to prevent cation dependent cell-cell interactions.

3. **Adherent Cells:**

Trypsin is usually used to detach cells from the plate surface and is neutralized with media containing FBS. The FBS re-introduces cations that aids in attachment to plastic and can cause cells to re-aggregate before sorting.

a) Use 5 mM EDTA or higher (**NOTE**: too much EDTA can kill your cells)

b) Accumax is cell dissociation solution that can aid in maintaining single cell suspensions.

4. **Samples with a High Dead Cell concentration:**

Dead cells can release their DNA into sorting media, which in turn can cause cells to clump together. Adding DNAse I in the presence of $MgCl_2$ will help reduce the aggregation.

a) Treat cells for 15-30 minutes in a sterile solution of 100 µg/mL DNAse I and 5 mM $MgCl_2$ in HBSS at room temperature.

b) Wash the cells 1x in HBSS containing 5 mM $MgCl_2$.

c) Re-suspend the cells in HBSS containing 25-50 µg/mL DNase I, plus at least 1 mM $MgCl_2$ prior to and during the sort (5 mM $MgCl_2$ is optimal).

<u>Sample concentration and minimum sample volume</u>

It is important to count the cells. Sorting speeds are limited by concentration and volume. **The nozzle size will be chosen by FCF staff**.

For a **bulk sort** (cells are sorted into 1.5 ml, 5 ml or 15 ml tubes), provide cells with following concentrations depending on the used nozzle:

Nozzle size	**Cell type**	**Concentration**
70 µm	Lymphocytes, small cells	40 x 10^6 cells / ml
85-100 µm	< 15 µm in diameter, cell lines	10-20 x 10^6 cells / ml

Please aim for a **final cell concentration** of 1-5 x 10^6 cells / ml for a **single cell** multi-well plate sort.

The **minimum sample volume** for cell sorting should exceed 200 µl. We can load samples in 1.5 ml Eppendorfs, 5 ml FACS tubes or 15 ml tubes.

Sample collection

The choice of **collection medium** depends on the future application and is variable. The only exception is made for harmful volatile substances like Trizol or 2-Mercaptoethanol, which are not allowed as collection medium in our facility.

The size of collection tube required depends on the amount cells you expect to retrieve:

- 15 mL conical tubes for large recoveries (one-way or two-way sorts only)
- 5 mL FACS tubes or Eppendorf tubes for smaller volumes (4-way sorts)
- Multi-well Plates (96, 48, 24, 12, 6)

Add media to the collection tubes to prevent the cells from drying out and dying. Use a concentrated media or have a high FBS content (20-50%) to help cells recover from the sorting process.

- 2-3 mL for 15 mL tubes
- 750 µL-1 mL for 5 mL tubes
- 100 µL for 96-well plate

Collection buffer:

The tubes/wells should be filled with an appropriate amount of sterile collection medium. In some cases, for example, RNA isolation, we can sort into lysis buffer (e.g. Qiagen RLT) into RNALater, or PCR mix.