Forschungszentrum für Immuntherapie
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CFFC PKZI Mainz Sorting Guide

The CFFC PKZI provides access to several high-speed cell sorters with up to 5 lasers and 18 colours; a BDFACS Aria II and a BDFACS Aria III SOPR. The correct Aria will be chosen according to your needs, as documented in the Sort-Request-Form.
In general, the Aria is booked to capacity, typically 1-2 weeks in advance.

Frequently Asked Questions (FAQ) regarding cell sorting

1. What should I re-suspend my cells in for sorting and at what concentration?

One of the most important steps prior to bringing your cells for fractionation is proper sample preparation. This is essential for obtaining good purity and post-sort recovery.

After the final wash, cells should be re-suspended at concentrations of approximately 40-60 million/ml (for primary cells) or 10 million/ml (for large, sticky cells and cell lines) in 5ml sterile polypropylene FACS tubes (Sarstedt, order number: 55.526.006). The sort media can be any buffered isotonic salt solution such as PBS, or Hanks Balanced Salt Solution (HBSS), **calcium and magnesium free** containing 10mM HEPES, pH 7.2.

You may also add 1-2% FCS, calf serum or BSA as most cells benefit from having some protein in the sorting media. However, serum concentrations higher than 2% can lead to cell aggregation and clogging.

For cell line samples, it is also recommended to add 1mM EDTA.

Do not use sorting buffers that contain phenol red as this causes an increase in background fluorescence and decreases sensitivity.

For sticky cells: Try increasing the concentration of EDTA to 5mM and use FBS that has been dialyzed against Ca/Mg++ free PBS or 0.5% BSA. Caution: Some cell types can be sensitive to high concentrations of EDTA.

For adherent cells: When using trypsin to detach adherent cells, use FBS that has been dialyzed against Ca/Mg++ free PBS or increase the EDTA concentration to 5mM or higher (first make sure that your specific cell type is not sensitive to high concentrations of EDTA). You may also try Accutase or Accumax, however Accutase can alter some surface epitopes and this effect will need to be determined empirically for the epitopes being evaluated.

For samples with a high percentage of dead cells: Cellular clumping can be caused by DNA released from dead cells. DNAse 1 in the presence of magnesium chloride will help to reduce cellular aggregation.
a) Treat cells for 15-30 min in a solution of 100ug/ml DNAse 1 & 5mM MgCl2 in HBSS at room temperature.
b) Wash cells once in HBSS with 5mM MgCl2.
c) Gently resuspend cells in HBSS with 5mM MgCl2& add 25-50ug/ml DNAse 1 (as a maintenance dose) prior to & during the sort.

Note: DNAse 1 requires at least 1mM Mg to work effectively, although 5mM is optimal. Actin released from dead cells irreversibly inhibits DNAse 1 so it is important to minimize the presence of dead cells.

2. How many cells can I bring?

This is the most commonly asked question. To answer it accurately, we require the following information:

a) What is the size of your cells and how fragile are they?
b) What is the approximate percentage of the population(s) you wish to sort?
c) How many sorted cells do you require in total for your downstream experiments?

2a. Cell size, type and fragility greatly influence the size of nozzle we use for sorting. Generally, the nozzle should be about 4 times the diameter of the cells to be sorted. For example, small round cells such as spleenocytes, lymphocytes or thymocytes require a 70um nozzle and fibroblasts require a 100um nozzle. Sorting will not be as quick with a 100um nozzle however. The right nozzle size with respect to your cells prevents clogging, increases purity and yield and leads to more stable side streams.

2b and c. The percentage of the population(s) you wish to sort along with the total sorted cells you require for post-sort experimentation are important factors in determining the total time needed for your sort (see question 3). For example, the subset you wish to sort is 10% of the total and you need 1 million cells for your experiment. In theory you would need to run 10 million cells through the sorter. However, the actual yield is typically 75-95% of this theoretical yield due to aborted events and the quality of your sample. Therefore it is recommended that you bring 25-50% more cells to the sorter than you would need if the actual yield were 100% (based on the abundance of your target cells).
Good sample preparation goes a long way to getting high post-sort recovery. Make sure you have a single cell suspension without clumps and as little debris and dead cells as possible. Clumps are removed by filtering the sample through a 70um mesh right before sorting. Adding EDTA and DNAse to troublesome samples will also help (see question 1).

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3. How long will it take?

This depends on many factors including the size and fragility of your cells, the concentration of your sample, the percentage of the population(s) you wish to sort and the total number of sorted cells required for downstream experimentation. The following table shows how many events are possible under different set up conditions. Each situation may be different, so these numbers should only be used as a rough guide. **Please note that the minimum booked time for the sorters is 1 hour, and this includes set-up time as well as a mandatory 20-30 minute post sort cleaning/sterilization procedure. Make sure you include extra time in your booking to include set-up and post-sort sterilization.**

Aria I, II or III

Nozzle	70µm	100µm
Cell Type	Lymphocytes 10,000/sec=36 million/h	Cell Lines 4000/sec=14.4 million/h

4. Do I need to filter my cells?

It is REQUIRED that all of your samples be passed through 70um nylon mesh, preferably right before running them through the sorter.

5. Should I use a dead cell exclusion dye?

Dead cells cannot always be entirely excluded by their light scatter characteristics. Therefore, it is highly recommended that you use a dead cell exclusion dye. Typically, we recommend a DNA-intercalating dye such as PI, 7AAD, DAPI, DRAQ7 or TO-PRO 3 Iodide. There are a wide variety of dead cell exclusion dyes available across the fluorescence spectrum. Please contact flow facility staff if you are unsure of the best dead cell dye to apply with respect to your experimental design.

6. How should I bring my samples to the lab?

Cells should be resuspended in 5ml polypropylene FACS tubes. Cells should be in an appropriate buffer that preserves both viability and controls pH.

7. What kind of tubes or plates should the cells be sorted into?

For abundant populations, you may sort into 5ml polypropylene FACS tubes or 15ml conical tubes. Please note that we can sort 4 populations simultaneously when using 5ml FACS tubes but only 2 populations simultaneously when sorting in 15ml conical tubes. Polypropylene FACS tubes are preferred over polystyrene as they build up less static charge during the sort resulting in a higher post-sort yield. For rare populations, it is best to sort directly into 1.5ml Eppendorf tubes. We can also sort directly into a variety of microtiter plates ranging from 6 to 384 wells, as well as onto slides. Please contact us in advance to communicate your requirements and we can advise you on the best choice for your experiment.

8. What kind of collection medium should the cells be sorted into?

We recommend 100% serum, HEPES buffered HBSS or MACS buffer for convenience.
We do not recommend sorting directly into TRIzol, or any other DNA/RNA extraction buffer, as the cells are contained within a drop of PBS which, with high sorted cell numbers, will significantly dilute your extraction buffer resulting in sub-optimal isolation. Sorters use PBS as its sheath fluid and mixing large quantities of PBS with buffer containing calcium chloride can produce a precipitate of calcium phosphate on the cell membranes. This can adversely affect cell viability. Therefore we also advise against sorting directly into culture medium. If you must sort into medium, keep in mind that the pH will rise with time so it is best to start with it on the acid side of neutral.

9. What kind of controls should I bring?

You should plan on bringing an unstained/negative control or untransfected/mock transfected, single stained compensation controls, along with FMO (Fluorescence Minus One) gating controls if performing a polychromatic flow cytometry experiment (over 5 colors). If you are using a dead cell exclusion dye, you must also bring a separate viability control. We recommend heat killing your cells at 65°C for 15 minutes and mixing with live cells followed by staining with your viability dye.

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10. How is the success of a sort assessed?

There are 3 factors that are important in determining the quality of a sort; **Purity, Recovery and Yield.**

Most users are primarily concerned with purity. In most cases, it is possible to achieve purities of 95% or higher. Purity is defined as the number of cells in the sorted tube that fall within your sort criteria. We can determine purity by taking a small aliquot and rerunning it on the sorter.

Recovery is defined as the number of cells in your sorted tube relative to the number of sorted cells recorded by the cytometer. This number should be 80% or higher, however there is always some cell loss due to cell death and/or not all sorted droplets landing in the collection tube.

Finally, yield is the percentage of cells you get back relative to the amount in your sample. Yield will be 70% or higher in most cases and is affected by the sort mode used. Unlike recovery, the cell loss estimated by the yield includes electronic and sort (hard) aborts and is also impacted by the dead volume in the sample line and particles that remain in both the sample line and sample tube.

Summary:

It is extremely important that the CFFC PKZI FACS Core staff receive feedback on the success of the sorts performed. We recommend that all users perform a post-sort cell count if possible in order to assess the quality of the sort performed. It is always best to contact the sort operator prior to your appointment if you are sorting with us the first time or are unfamiliar with sorting cells.