Guidelines for cell sorting experiments - RPCI

Welcome to the RPCI Flow Core Sorting Facility. We are looking forward to helping you achieve the best results for your sorting needs. These guidelines should help you understand what is involved with obtaining a great sort result at our facility.

Our facility currently operates two cell sorters. Our Aria I is configured similarly to the LSRIIB analyzer with a violet/blue/yellow-green/red laser combination, but with only 2 options off the violet laser. The Aria II is configured with a UV/violet/blue/red laser combination similar to the Fortessa B analyzer, but with only 3 options off the violet laser. The instruments have comparable capabilities otherwise.

We operate the sorters with dedicated staff personnel, so that individual researchers will not have to learn the technical operation details of these complex instruments. However, for individuals who would like to learn how to operate the sorters by themselves, for instance to allow for lengthy after-hours sorts, we can provide a detailed training.

Scheduling and reservations:

New investigators are asked to schedule an appointment to discuss their sorting needs with the sorting staff. We can advise you on the best approach to get you the purity and yield that you require, as well as address potential biosafety issues.

Sorts can only be scheduled by the sorter operators (Kitty x8416, Xiaojun x1745), although current bookings can be viewed at our calendar website (https:/fomweb.roswellpark.org/fom) after you register on this system. On regular business days, our sorters are typically ready to sort by 10am and can run until 6pm. Appointments past this time will be possible after prearrangement with the operators.
For each sort appointment, please fill out a Sort Request Form at the time the reservation is made (see instructions below). The completed information helps us understand the expected duration and instrument requirements of your sort (lasers, nozzle, and pressure to use). The Aria sorters can get booked well ahead of time, so please keep this in mind when scheduling. Same-day or next-day appointments can rarely be accommodated.

At the start of each sort, we analyze the sample(s) and review the sort logic with you, to make sure that everything is set up how you want it. It usually takes about 15-30 minutes to pre-evaluate your sample and set up or adjust the instrument parameters. We ask that you plan on staying for that period of time so that we fully understand your sorting goals. Once the set-up is completed, we will operate the sort for you. A typical sort rate is 2,000-5,000 events/sec, or 7-18 million cells/hour. You can use these criteria to estimate how much time your sort will take.

Cancellation policy and Charges:

A valid account in FOM is required for all sort appointments. All appointments are charged at actual sort time (billed in FOM) OR 80% of the reserved time, whichever is greater. In addition, a one-hour setup charge is added to each daily use per instrument. This charge covers the setup of the instrument,

including routine maintenance, cleaning, filling sheath, and QC. This charge does not include experiment setup (such as adjusting voltages and gating), which will happen during the appointment.

Appointments cancelled more than 1 week in advance will not be charged. Appointments cancelled within 7-3 days will be charged for 50% of the reserved time. Appointments cancelled within 48 hours of the scheduled time will be charged 80% of the reserved time. “No shows” will be charged for 80% of the reserved time plus the 1-hour setup fee.

Reserved time will be charged at 80% of total time, even if not used. Any exceptions to this will be at the discretion of the operator and/or manager. Proper submission of the sort request form and a detailed discussion of the projected sort duration with the operators can avoid disputes pertaining to reservation time. This applies to reservations that are underestimated and take significantly longer than expected (that can interfere with a next user’s time slot or closing hours), as well as those that are overestimated (resulting in sorter idle time and incurring charges for unused time).

Filling out the Sort Request Form:

The form can be found on our website or we can send a Word version. The form is to be filled out as completely and accurately as possible for each appointment. A valid grant number (RPCI) or PO number (UB and other external users) as it appears in your FOM account is mandatory.

The number of samples and the estimated total number of supplied cells need to be filled out accurately so that we can correctly book the time allotted to your sort. For instance, if your Sort Request Form indicates 2 samples with 20 million cells each, but instead you bring 4 samples with 50 million cells each, we may not be able to accommodate you.

Please indicate which exact fluorochromes are in your samples. If you do not see the fluorochrome that you want to use, it is not a standard option and needs to be written in under “Other”. If uncertain, please contact us to see if we have the right laser/filter set available for the fluorochrome that you want to use. Be precise: For instance, if your fluorochrome is RFP, do not select PE. This greatly helps us identify which instrument will be best for your experiment, and allows us to do much of the experimental set-up ahead of time.

Part of the instrument set-up is to choose the correct nozzle size and pressure for the sorter. If you are not familiar with this technical detail, please indicate on the form exactly what kind of cells (and in particular how large) you will bring, and whether you are concerned about cell fragility.

Biosafety:

Our Aria instruments are in a BSL2+ facility, and thus all users and people entering the room are required to wear proper PPE. Lab coats are mandatory, and surfaces are only to be touched with gloves. Personal devices, food, drinks, and cosmetics are not permitted in the room.

Special precautions are in place on the Aria I to run samples infected with specific pathogens that require BSL2+ level precautions. These sorts can only be performed after prior discussion with and approval of the facility direction. Radioactively labeled samples are not permitted in our entire facility.

Samples:

To get an estimation of how many cells you need to sort, assume that you will get a yield of 50% of the targeted population. Our sorting efficiency is typically 70 – 90%, but there will be some further loss due to the mechanics of the instrument that can put stress on the cells.

Ideally samples are supplied at a concentration of 10-20 million cells/ml. We will dilute if necessary, so please bring appropriate buffer if you need anything else but standard PBS for the dilution buffer. Remember that diluting a sample is easier than concentrating it, although we do have a centrifuge available if needed. For small cell numbers, we suggest supplying the cells in at least 0.5 ml buffer or medium. If medium is used, it needs to contain less than 2% FBS (serum), and ideally it is free of phenol red. You can supply samples in any type of tube, but we will need to transfer them to 12x75mm style tubes prior to sorting.

Samples must be filtered (<40 µm mesh) prior to sorting to prevent clogging of the instrument. We routinely use 35 µm nylon mesh cell strainers for all samples that are not previously filtered or that require resuspension from a pellet prior to sorting. We may also re-filter samples when they start clumping during the sort. Because these filter tubes are expensive, we expect you to filter your samples prior to bringing them to us, and we reserve the right to surcharge for the filter tubes if we need to supply them.

To prevent clumps in your sample due to release of DNA if lots of dead cells are present, it is recommended to add 10 U/ml DNase (in presence of 1-5 mM magnesium). Instead, for some samples it can help to use EDTA-containing buffer to prevent aggregation.

Sample collection:

We can collect the sorted cells in several ways. Our typical setup is a four-way sort into 12x75mm tubes, which can sort out 1 to 4 different populations simultaneously from your original sample. You can choose from polystyrene, polypropylene (material same as microfuge tubes), or glass tubes (12x75 mm). The same collection device can be used for Eppendorfs and PCR tubes with some restrictions. For two-way sorts of larger cell fractions, we use 15-ml conical tubes or 16x100 mm glass tubes. We can supply the (sterile) tubes, but you are encouraged to provide them yourself (prefilled with collection buffer). The Aria instruments also have the option to sort on plates (384-, 96-, 48-, 24-, 12-, or 6-wells). These will have to be supplied by you, and they need to be BD brand plates for a proper fit in the instrument.

The collection tubes need to have a small amount of collection buffer or medium (~10 – 20% of the tube volume) to cushion the cells as they are deposited at high speed into the tubes. This can be your growing medium or anything else that you would like to use. It is recommended to include FBS (serum) or BSA to coat the tubes with a cushioning layer prior to sorting. If you are going to grow sorted cells, collecting into straight FBS is usually foolproof. Keep in mind that the tubes will be filled further with our sheath buffer, which currently is a Hepes-based isotonic buffer that contains 1 mM EDTA. The volume added to the tube will be proportional to the number of cells collected: 1 million cells will add about 2-3 ml volume to a tube dependent on the setting.

What to bring for your sorting experiment:

1. Unstained cells. If this is the first time you do this experiment, make sure to bring a large quantity of these (>100,000 cells in at least 500 ul), as we use them to adjust the instrument settings for size scatter and fluorescent background of your cells.
2. Single stained Compensation controls – for each fluorochrome that you are using. These can be either the cells from your experiment labeled with each fluorochrome separately, other cells that have significant staining with each separate fluorochrome, or they can be commercially available compensation beads labeled with your antibodies.
3. FMOs and other controls as needed.
4. The filtered sample(s) to be sorted. We will need to run some of this sample for a presort analysis, so that the gating strategy can be defined.
5. Dilution buffer for the samples.
6. Collection medium, preferably already aliquotted into collection tubes, plus some extra.
7. Extra collection tubes.

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