Guidelines for Cell Sorting
University Flow Cytometry Resource
University of Minnesota

Updated: 11/21/22 JAM

Procedure:

1) Fill out a **New Sort Customer Form**. (New Customers Only)

2) Fill out a **Biosafety Form**. (New Customers and New Applications)

3) You will be contacted to schedule a pre-sort experimental consultation with the sort operator. (New Customers Only)

4) Consult the **Cell Sorters Online Calendar** to select a date and time frame and fill out the **Cell Sorting Request Form**.

5) You will receive an email containing your sort details when it is scheduled or you will be contacted if UFCR staff has questions or your reservation cannot be scheduled.

Note: After becoming a registered customer, sorts may be scheduled by following the directions in Step 4. The request form should provide information to allow for pre-entry of important information for the sort. Last minute changes cause delays in setup, which will translate into lost, non-refundable sort time.

What we need to know before each sort:

Biological or chemical hazards: Sorters generate aerosols that can be greatly exacerbated by clogs and expose the sort operator to known or unknown chemical and biological hazards. We need to be informed of the exact nature of the cells and their treatments/stains/dyes that will be run through the sorter. This should be described **in detail** in the Biosafety Form.

Cell size, total cell number and nozzle size selection: We expect you to know the number of cells you will be bringing and their approximate size in suspension. This information allows us to select the correct nozzle size, which will give you optimal resolution, decrease the chance of spraying sort streams and of creating instrument clogs.

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Cell adhesivity: We need to be provided with a filtered single cell suspension to avoid splashing side streams or clogs, which create potentially dangerous aerosols. This is no small task for adherent cell lines but we do expect you to optimize it to the best of your ability. Talk to your operator about ways to obtain the best single cell suspensions.

Sample controls: You should have this discussion with the sort operator during the pre-sort consultation or prior to your sort. You will need at a minimum: an unstained sample and single stained controls for each fluorochrome. Other considerations include experimental controls and FMO controls for gating dim or rare populations.

Cell concentration: We suggest 2.5 x 10^6 cells/mL (130µm nozzle), 10.0 x 10^6 cells/mL (100µm nozzle), 15.0 x 10^6 cells/mL (85µm nozzle) and 20.0 x 10^6 cells/mL (70µm nozzle) in a maximum sample volume of 3.5mL for the 5mL tubes or 10mL maximum for the 15mL tubes.

Sample tubes: They should be polypropylene or polystyrene 5mL tubes or 15mL tubes. All samples must be submitted with covers or caps. Appropriate tubes include 5mL polystyrene test tubes (Corning Falcon™ catalog number 352054).

Cells must be filtered: Use 5mL polystyrene test tubes, 12 x 75mm with cell strainer cap (35µm) (Corning Falcon™ catalog number 352235) or for larger volumes of sample use a mesh filter with pore sizes no larger than 40µm.

Sample media: PBS or RPMI based with 0.1%-2.0% BSA or HSA or 2.5% FBS. Use of 1mM-5mM EDTA or 25-50µg/mL DNase to reduce aggregates is strongly recommended. Other variables to consider are the presence or absence of Ca^{++}/Mg^{++}, trypsinization and quenching steps. We can give you some recommendations if you want them.

Catch tubes: They should be sterile polypropylene or polystyrene Eppendorf, 5mL or 15mL tubes, slides or 6-96 well plates. Catch tubes should be coated with a protein-based media (ex. PBS with 25% FBS if anticipating filling the catch tube) on the inside and have at least the catch media volumes specified below.

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Catch tube media volume:

Catch Tube/Plate	15mL	5mL	1.5mL	96-well	48-well	24-well	12-well	6-well
Catch Media Volume	3mL	1mL	300µL	100µL	250µL	500µL	1mL	2mL

Sample recovery expectations: Target population recovery depends on a number of factors including the percentage of the estimated target population and the relative efficiency as determined by the sample. Variables such as how delicate and how sticky your cells are and sample concentration can all impact yield and purity. In terms of speed, there are frequently large tradeoffs between increasing the flow speed and viability, purity, resolution and yield. Faster is not better. There is an optimum setting depending on the nature of the sample, which will result in the best achievable yield, purity and sensitivity. Excessive debris and unlysed RBCs will impact yield. Sorting is like any other scientific methodology – it requires optimization for best results. We highly encourage your first sort to be treated as a pilot study to determine actual recovery and ways to improve sample preparation.

Particle/cell size, sample throughput and sample concentration ranges for available nozzles:

Nozzle	**Particle or Cell Size**	**Expected Sort Rate (Events per hour)**	**Sample Concentration**
70µm	1µm–10µm	10 – 30 million events per hour	20-40 million cells/ml
85µm	8µm-18µm	10 -15 million events per hour	10-15 million cells/ml
100µm	15µm-23µm	5-10 million events per hour	5-10 million cells/ml
130µm	24µm-32µm	2-4 million events per hour	2-5 million cells/ml

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Online Resources:
Relevant Webpages: **University Flow Cytometry Resource Home** (flowcytometry.umn.edu), **Cell Sorters**, and **Cell Sorting Services**.
Relevant Links: **Biosafety Form**, **Sort Request Form** and **Instrument Calendars**.

Facility locations and phone numbers:
Masonic Cancer Research Building Room 695 (MCRB 695)
Phone: (612) 624-7680
Cancer and Cardiovascular Research Building Room 1-209A (CCRB 1-209A)
Phone: (612) 624-7680.
Microbiology Research Facility Building Room 2-128A (MRF 2-128A)
Phone: (612) 624-7680.

How much time do you need to book?

Aseptic setup: 60 Minutes (Includes nozzle calibration and QC; Select Aseptic Setup: Yes in Sort Request form). We will automatically add this procedure before your requested sort start time.

Nozzle calibration and QC: 30 minutes. We will automatically add this procedure before your requested sort start time.

Experimental setup: This is the time needed to create everything for your experiment and can be used to generate a template for future use. The basic outline for an experiment (sample creation, setting voltages, worksheet set-up, etc.) will take between 5-15 minutes. A similar amount of time is needed to verify the template for future experiments. Compensation takes roughly 5 minutes per fluorophore in your panel and with a good template may not have to be done every sort. 5 minutes is needed to clean the instrument between samples. After your population(s) is collected, a post-sort can be run. This takes 5 minutes for every population sampled.

Cleaning and data transfer: This will be performed during the last 15 minutes of your requested sort.