Guidelines for Cell Sorting
University Flow Cytometry Resource
University of Minnesota

Updated: 11/13/15 TM/PEC

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 Instrument Reservation Calendar to select a date and time frame and fill out the online Cell Sorting Request Form
5) You will receive an email containing your sort details when it is scheduled or you will be contacted if 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 can cause delays in setup, which will translate into lost, non-refundable sort time.

What we need to know before you sort:

Biological or chemical hazards:

Sorters generate aerosols which can be greatly exacerbated by clogs and fluid stream aberrations. These also heighten exposure of the sort operator to chemical and biological hazards present in the sample. All hazards present in a sample should be disclosed to UFCR staff whenever applicable. This should be described **in detail** in the Biosafety Form.

Cell size and total cell number:

Knowledge of the approximate cell size or size range
This information allows us to select the correct nozzle size, which will:
1) provide optimal signal sensitivity
2) decrease the chance of spraying sort streams
3) decrease the chance of clogs
4) increase purity

Cell adhesivity/aggregation:

Please provide a filtered, single cell suspension to avoid unstable sample flow or clogs, which create potentially dangerous aerosols. This is challenging for some samples such as adherent cell lines and diffcult tissue samples. Sort purity can be significantly impacted by aggregated samples. Contact UFCR staff for more information regarding methods to obtain the best single cell suspensions.

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Sample Preparation

Controls:
Please discuss this with the sort operator scheduled for your work. You will need at a minimum: an unstained sample, single stained controls for each fluorochrome. Other considerations include experimental controls, viability stains and Fluorescence Minus One(FMO) controls for gating dim or smeared populations.

Cell Concentration:
We suggest 2.5 x 10e6 (130um nozzle), 10 x 10e6 cells/ml (100um nozzle), 5.0 x 10e6 cells/ml (85um nozzle) and 20.0 x 10e6 cells/ml (70um nozzle) in a maximum sample volume of 3.5ml for the 5 ml tubes or 10 ml maximum for the 15 ml tubes. Err on the side of high concentration and bring additional sample buffer to the sort appointment for dilutionSplit sample into multiple tubes if necessary.

Sample tubes:
Sterile polypropylene or polystyrene 1.5 ml, 5 ml tubes, or 15 ml tubes. All samples must be submitted with solid covers or caps.
5 mL polystyrene test tubes,12 x 75-mm; 125 per bag (Falcon™) catalog number 35-2054(U Market available)

Cells should be filtered:
5-mL polystyrene test tubes, 12 x 75-mm with cell strainer cap (35um) 25 per bag (Falcon™) catalog number 35-2235 or for larger samples a mesh filter with pore sizes no larger than 40um.

Sample Media:
Final sample suspension should be performed using a buffered solution such as PBS, RPMI or other solution to maintain cell integrity during the entire sort and transportation periods.
Protein from 0.1%-2.5% BSA or HSA or 2.5% FBS is also recommended.
Other variables to consider are the presence or absence of Ca++/Mg+, trypsinization and quenching steps, addition of EDTA or DNASE. Please contact the UFCR for further assistance in sample media suggestions.

Catch/collection tubes:
Sterile polypropylene or polystyrene 1.5 ml, 5 ml tubes, 15 ml tubes, slides or 12-384 well plates. Catch tubes should be coated with a protein based media on the inside and have a specified minimum amount of media(see below).

100 ul in a microfuge tube or 96-well plate
0.5-1ml in 5ml 12 x 75 tube
1-4ml in 15ml conical tube

1ml in a 12 well plate; 0.5ml in a 24 well plate; 250ul in a 48 well plate

Sample recovery expectations:

Sorting is similar to other scientific methodologies – requiring optimization for best results. We highly encourage your first sort to be treated as a Pilot Study to determine recovery and ways to improve sample preparation.
Target population recovery depends on a number of factors including the estimated target population percentage and the relative efficiency as determined by the sample and instrument.
Variables such as cellular robustness, aggregation, cell concentration and sample flow rate can effect signal resolution, viability, yield and purity. Increases in sample flow rate above an optimum level for the sample and instrument setup may have an impact on viability, purity, resolution and yield. Faster is not always better. Excessive debris and unlysed RBC's will effect yield.

How much time do I need to reserve?

Procedure	Time to complete
Aseptic Set up	60 Minutes
System Setup, Calibration and QC	30 minutes or as a part of the 60 minutes aseptic set up time
Software Set-Up	15-30 minutes. Involves creation of an experiment and attributes within the sort software. Time reduced if a previously validated template is used.
Voltage and Compensation adjustment	3-5 minutes per fluorochrome
Gating strategy(Worksheet design)	5 minutes
Sample concentration adjustment	3 minutes
Sample Run Time	nozzle dependent; see table below for averages
Sample exchange	2-3 minutes
Post sorts	3-5 minutes for each population
Instrument Cleaning	15 minutes
Data Transfer	15 minutes

Note: Clogs resulting from poor sample prep will be resolved on customers booked time and is non-refundable. We highly advise the use of DNASE or other sample quality-enhancing techniques whenever possible. The UFCR will

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Nozzle	Particle Size Guide*	Events per second	Events per hour**	Concentration
70um	1 – 10 um	5,000 -10,000	15 - 30 million events per hour	20-40 million cells/ml
85um	8-18 um	2,500 – 5,000	10 - 15 million events per hour	10-15 million cells/ml
100um	15-23 um	1,200 – 2,500	5 - 10 million events per hour	5-10 million cells/ml
130um	24-32 um	500 - 1000	2 - 5 million events per hour	2-5 million cells/ml

* Size is that of the cells being sorted

** Events are defined as all those detected above threshold. Knowledge of the sample contents is needed to accurately assess the time required to run the sample through the cytometer.

Online Resources:

Please visit the link below for more information on our cell sorting policies and procedures.
http://www.health.umn.edu/research/resources-researchers/flow-cytometry-resource/cell-sorting

Facility Locations and phone numbers:

Masonic Cancer Research Building 695 (MCRB); 612 624-7680

Cancer and Cardiovascular Research Building 1-209A (CCRB); 612 624-7680

Microbiology Research Facility 2-128A (MRF); 612 625-3653