Information for Submitting Cells for Cell Sorting at Flow Cytometry Lab

Penn State University – Huck Institutes of the Life Sciences
Location: W-124A Millennium Science Complex
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***Please read through this document and contact us with any questions before your sort.

Scheduling:

Sorting can be performed between the hours of 10:00am-4:30pm. If sorting outside of the regular hours is required, such a request must be submitted to Mitch Koptchak (mrk226@psu.edu) and Rajeswaran Mani (rpm5900@psu.edu) and approved. **A minimum of 30 minutes between sorts is required to reset and clean the instrument.** ***If a different nozzle needs to be used, a minimum of 1 hour between sorts is required.***

Scheduling of appointments on the sorter can be done by emailing Mitch Koptchak (mrk226@psu.edu). Sorting must be scheduled a minimum of 1 working day before the requested appointment time.

The Astrios:

The Beckman Coulter MoFlo Astrios is contained inside a biosafety hood and uses either a 70µm or 100µm nozzle tip for most standard cell sorts. **However, due to limitations to our in-house air pressure and potential cell damages associated with high pressure we typically discourage customers from utilizing the 70µm nozzle.**

Typically, cells that are **larger** than 10µm, such as:

- primary cells isolated from tissue
- immortalized cell lines
- primary cells stimulated in-vitro

cells that are sensitive to high-pressure (high pressure = 60psi), including stem cells & dendritic cells are best sorted at low pressure (20-25psi) with the 100µm nozzle. This approach maximizes recovery and viability, and minimizes nozzle clogs

Cell Concentration:

Maximum concentration: cells >10µm, 5 x 10^6/ml cells <10µm, 10-20 x 10^6/ml
Cells should be counted after all sample staining and other preparation as is not uncommon to lose up to 50% of cells during the staining process. At ideal concentrations, we can operate the cell sorters at their most efficient event rates; with 100µm tip at 10,000-12,000 cells/sec (approx. 30-42*10^6/hour). If your cells aggregate at high concentrations, adjustments to concentrations may be needed. Please bring extra buffer along so that we can dilute the sample if we observe

excessive aggregation. Cell lines and other samples with non-uniform size and shape frequently require a lower concentration than the maximum listed above.

Minimum Volume: (500-750µl)

Cells should be a **minimum volume of 500-750µl** even if that volume does not give the ideal cell concentration described below. This is because we need to run a small volume of cells before sorting to analyze the sample and set sort gates. We want to use as little of your samples as possible to do this.

Staining Large Amounts of Cells for Sorting:

When staining large numbers of cells, the antibody concentration rather than the cell number is the important factor. If you are staining 10 million cells, adjust the antibody amount accordingly. If you are staining 100 million cells, increase the antibody 5-10 fold.

Sorting Sample Buffer

Basic Sorting Buffer

*** 1x PBS (Ca/Mg++ free) ***
*** 1% FBS or BSA ***
1mM EDTA
25mM HEPES pH 7.0

* Culture media is not ideal for sorting for the following reasons:

- Cells should always be in Ca- Mg- PBS/FBS
- The pH becomes basic under normal atmosphere reducing the cell viability.
- The **calcium chloride in most culture media is incompatible with the phosphate component of the instrument sheath buffer causing calcium phosphate crystals to form.**
- The phenol red increase the background fluorescence of the cells which may reduce the resolution between negative and positive cells.

Sample and Collection Containers:

Sort collection tubes should be polypropylene 5ml – 12x75 round bottom tubes which suit our instruments the best, however, 15 and 50ml tubes and 1.5ml snap-tops can also be accommodated. Single (or multiple) cell sorts into tissue culture plates are also an option.

Sorting Populations:

We can sort up to 6 populations simultaneously on the Astrios. Though sorting 6 mutually exclusive populations is the most efficient, overlapping populations can also be sorted in order of importance. 3-6 way sorting can **ONLY** be done into 5ml tubes or smaller.

Collection Buffer:

Collection containers should have **some type of buffer in them for the cells to fall into**. It is recommended it be 0.5ml - 1ml per 5ml of collection volume: i.e. 1ml in a 5ml tube.

The following can be used:

- PBS if collecting cells for RNA or DNA
- Fetal Bovine Serum only for culturing cells

50% serum is a good starting concentration for collection as this will be diluted down by the sorted cells which are delivered in a droplet of sheath fluid. For the 70um tip, 1 million collected cells will add about 1.5 mL sheath fluid; for the 100um tip, 1 million collected cells will add about 3.5mL sheath fluid.

Sort Efficiency:

You should plan on a final yield of about 75% of the starting number of desired cells. For example, 1 x 10^7 cells with 30% positive for GFP would yield 2.25 x 10^6GFP positive cells)10^7 *0.3*0.75). Keep in mind that "clumpy" cells produce a greater number of doublets, which can reduce this yield. **Rare event** sorts (1% or less) can produce a lower yield, as low as 50%.

Sterile Sorts:

We perform sorting in an aseptic manner. The instruments are cleaned between sorts using 10% bleach solution and sterile water. In addition, sheath fluid goes through a 0.04µm in-line sheath filter.

Problems:

Sticky Cells:

Cell sorting requires cells in a single cell suspension. If the cells are clumped, they cause **MAJOR** problems.

- A large clump will clog the cell sorter which causes a delay and may contaminate the collection tubes
- Clumped cells will also reduce the sort yield due to the clumps failing the single discrimination gating
- Aggregated cells cause more coincidence (or software) aborts

We can filter samples using sterile 35 micron filters before sorting **($1 per filter**).

Cells sticking to the sides of collection tube may also affect your cell yield. Precoating the collection tubes with fetal bovine serum helps to prevent this.

Frequently dead, lysed cells, especially neutrophils, cause severe clumping problems. If this is a problem, it is helpful to stain on ice and use a DNase cell staining buffer) EDTA should be avoided for the DNase to work properly!)

Staining Buffer with DNase
PBS with 1% serum albumin
100 units/ml DNase I
1mM MgCL2

Adherent cell lines can reaggregate when serum is used to inactivate trypsin. Soybean trypsin inhibitor can be used as an alternative to serum. Each cell type responds differently to SBTI and the ideal conditions must be determined empirically, but usually between 0.05%-0.25% SBTI in TBS (1-2mL per 25 cm^2 surface area) will work.

Purity:

If cell numbers permit, a small amount of cells should be reanalyzed to verify accuracy of the sort. Factors which negatively affect purity include the following:

- Clumped cells
- Dim fluorescence
- Low percentage of cells in the sort gate

Poor Viability:

If sorting more than one or two samples, it is best to stagger the cell preparation process so that the cells are not stored at less-than-ideal conditions for any longer than necessary.

Optimizing Cell Sorting:

Sorting can be optimized to provide greater cell recovery at the expense of purity or purity at the expense of recovery. It may take several sorts to optimize the sample preparation and sorter operation for your cells, but we find that once optimized sort results are highly consistent.

Biohazard Policy:

All Principal Investigators who desire to sort cells using the Flow Cytometry Lab will be asked to submit a **Sorting Request Form** detailing the sample types and biohazard testing specifications which will be used for the project. One form can be submitted to encompass an entire project while the related sorting form will be required for each sort appointment.

Regulatory standards require that cell sorting laboratories comply with the BSL2 requirements at a minimum. As cell sorting is known to create aerosols, many organisms which can normally be safely handled at the BSL2 level require "BSL2 with enhanced precautions" due to the potential creation of aerosols while sorting. The Flow Cytometry Lab is able to comply with these standards up to the level of BSL2 with enhanced precautions. **The Lab will not sort samples which require BSL3 containment.** Organisms requiring BSL3 containment include influenza (1918,Avian, H1N1), COVID, Monkey pox, Mycobacterium tuberculosis, Mycobacterium bovis, Neisseria meningitides, Treponema pallidum, measles virus, Coxiella burnetii (Q fever), or any other organism which may be transmitted by aerosols.