# Submitting Cells for Cell Sorting

Penn State University – Huck Institutes of the Life Sciences
Microscopy & Cytometry Facility: **Flow Cytometry Lab**
**Location:** W-124A Millennium Science Complex
**Contact Us:** l-flow@lists.psu.edu or bcd5018@psu.edu
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**Please read through this document and contact us with any questions before your sort.**

## Scheduling:

A calendar showing available/busy times for the cells sorter is available for authorized users of RIMS (see rims.psu.edu). 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 Sarah Neering (sjn16@psu.edu) and approved. **A minimum of 30 minutes between sorts is required to re-set the machine.** ***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 **calling or emailing (preferred).** 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 biosaftey hood and uses either a 70um or 100um nozzle tip for most "standard" cell sorts – please contact us for more information about additional tip sizes.

Typically, cells that are **larger** than 10um, 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 100um nozzle. This approach maximizes recovery and viability, and minimizes nozzle clogs.

Cells that are **smaller** than 10um, such as:

- whole blood
- bone marrow
- splenocytes
- lymph nodes

can be sorted using the 70um nozzle at high-pressure (60psi) since the size is less than 10um. **Exceptions** would include human B-cells, cells sensitive to high-pressure, or samples with a low total number of cells to sort (<40 x 10^6).

## Cell Concentration:

Maximum concentration: cells >10um, 5 x $10^6$/ml  cells <10um, 10-20 x $10^6$/ml

Cells should be counted after all sample staining and other preparation as it 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: the Astrios with 70um tip at 25,000-30,000 cells/sec (approx. 80-100*$10^6$/hour), and with 100um tip at 10,000-12,000 cells/sec (approx. 30-42*$10^6$/hour). If the cells are less concentrated, it will take longer to sort through your samples although the final yield of desired cells will be about the same. In other words, your fees will be much higher than they need to be. If your cells aggregate at these high concentrations, adjustments to concentration may be needed. Please bring extra buffer along so that we can dilute the sample if we observe excess aggregation. Cell lines and other samples with non-uniform size and shape frequently require a lower concentration than the maximum listed above.

**Minimum Volume:**

Cells should be in a minimum volume of 400 µL 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, use the same staining volume and antibody amount that you use when staining 1 million cells. If you are staining 100 million cells, increase the antibody 5-fold.

**Sorting Buffer:**

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

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

- 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 increases 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 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 are able to 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 Media:**

Collection containers should have some type of media in them for the cells to fall into. The general rule is to have 1ml of media per 5ml of collection volume: i.e. 1ml in a 5ml tube.

The following media can be used:

- Culture media with antibiotics
- PBS if collecting cells for RNA or DNA
- Fetal Bovine Serum only

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 1.5mL 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 $10^6$ GFP 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:**

While absolutely sterile sorting is not technically possible, most of the sorts we perform are done in an aseptic manner with no resulting contamination. The instruments are cleaned between sorts using sterile Dullbecco's PBS. In addition we use sterile sheath fluid which goes through a 0.04um in-line sheath filter

**Problems:**

**Sticky Cells:**

Cell sorting requires cells in a single cell suspension. If the cells are clumped they cause several 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 singlet discrimination gating
- Aggregated cells cause more coincidence (or software) aborts.

We can filter samples using sterile 40 or 70 micron filters before sorting ($1 per filter).

Cells sticking to the sides of the collection tube may also affect your cell yield. Pre-coating the collection tubes with fetal calf 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 (for human samples, be sure to use human serum albumin!)
100 units/ml DNase I
1 mM 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 PBS (1-2mL per 25 $cm^2$ surface area) will work.

**Purity:**

If cell numbers permit, a small amount of cells are reanalyzed to verify the 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 project questionnaire detailing the sample types and biohazard testing specifications which will be used for the project. One questionnaire 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 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), Monkeypox, Mycobacterium tuberculosis, Mycobacterium bovis, Neisseria meningitides, Treponema pallidum, measles virus, Coxiella burnetii (Q fever), or any other organism which may be transmitted by aerosols. Material Safety Data Sheets (MSDS) on many infectious microorganisms are available at the Health Canada website, http://www.phac-aspc.gc.ca/lab-bio/res/psds-ftss/index-eng.php