Guideline for Fluorescence Activated Cell Sorting (FACS)
Fluorescence Cytometry Core
Center for Environmental Health Sciences
University of Montana

A consultation with the Core Operator should be scheduled before all sorts.

Things to consider before sorting:

- **Size:**
  - The cell size should not exceed one-fifth of the nozzle diameter.
  - Too large of a particle in a drop will alter the droplet break off resulting in fanning (missing the catch tube) or changing the break off (compromises sample purity.)
  - Clogs will happen if the particle or clumps of particles are greater than the nozzle size.
  - Nozzle size guideline:
    - 5-15 um = 70 um nozzle
    - >16 um = 100 um nozzle

- **Morphology:**
  - Cells that deviate from an ideal spheroid shape are more susceptible to shear induced damage and cell death.
  - Even if you have a small cell, switching to the larger (100 um nozzle) often results in better viability.

- **Cell Adhesiveness:**
  - Cells that stick together are problematic for sorting. Clumps will cause clogs, or your sort will contain contaminates of unwanted cells.
  - Using a bit of EDTA or careful trypsinization of cells often helps.
  - Using a slightly lower concentration than normally suggested for sorts will also sometimes mediate unwanted clumping.
  - Careful filtering and re-filtering during sorting is often necessary. Be sure to add additional time and total cell numbers for adhesive cells.
  - Be aware that any clogs will cost a lot of (billable) time.

- **Cell Fragility/Physiology:**
  - Fragile cells or cells that have been stimulated or otherwise altered from their normal resting state can have decreased viability after a sort.
  - Using a lower stream pressure can sometimes make a smoother ride for the cell. Please let the Core Operator aware if you suspect fragile cells.

- **Concentration:**
  - For most cells an average rule of thumb is 5-10 X 10^6 /ml. For fragile cells, adherent cells or large cells use a bit more dilute concentration.
  - It is better to bring the cells on the higher concentrated side along with some extra sorting buffer to dilute if necessary.

- **Sorting Buffer:**
  - Basic Suggested Recipe:
    1X Phosphate Buffered Saline (Ca/Mg^{++} free)
    1 mM EDTA
    25 mM HEPES pH 7.0
    1% Fetal Bovine Serum (Heat-Inactivated)
    0.2 um filter sterilize, store at 4° C
  - For sticky cells try increasing the EDTA to 5 mM

- For samples with a large number of dead cells, try adding 10 U/ml DNAase to reduce the clumpiness caused by DNA coating the cells.
- Do not bring cells in culture media.

- **Collection Media:**
  - Media that will keep cells viable/'happy' after being diluted with Sheath fluid.
  - Consider protein concentration, media type, if Ca^{++}/Mg^{++} needs to be added back in, and your final goal for your cells. (Antibiotic?)
  - Amount: typically about 1 ml for the 15 ml Conical Catch tubes, 0.5 – 1 ml for the 5 ml Falcon tubes and 100 ul in Eppendorfs or 96 well plates.
  - Please bring extra media and tubes/collection plates.
  - It helps to protein coat tubes to prevent sticking to the tube.

Frequently Asked Questions:

- How many cells should I bring? How many will I get back?
  - This is a complex question with a short answer. Bring approximately twice the number of cells you expect to get back based on frequency. For example if you have 25% frequency in your presorted cells and you want $1X10^6$ cells, you need to bring $8X10^6$ cells.

- Why so many? Where do they all go?
  - A lot of things are in play here. If your sample is highly concentrated you will lose a lot of cells to "coincidence" aborts. If a desired event is too close to a non-desired event, the drop will be aborted. Often times cell counts are done before the multi-steps of staining, washing, and resuspending and many cells can be lost. Filtering your samples will also lose some of your cells. You also need to account for the last bit in the tube that cannot be acquired or the cells that stick to the side of the presort tube.

- How long will it take?
  - Another question, not easily answered. Again, many factors will determine sort speed – concentration, volume of cells, sheath pressure, frequency of desired events, level of purity desired, complications due to clumps, clogs, etc. Although the instrument is advertised as being able to sort 25,000 events/second that only happens with small, durable, easily discerned populations (ie lymph subsets.) As a general rule, you take your frequency (% of cells in presort sample) times the event rate (a more realistic rate is 6,000 – 10,000/sec) to get the number of sorted cells per second. Then you extrapolate how much time will be needed to get the yield you hope for. With the above example of wanting $1X10^6$ cells of a 25% frequency you would take .25 X 8,000 events/sec to get 2,000 events/sec or 7.2 X 10^6 per hour, so you could easily get your yield in less than 15 minutes. However, if you need $1X10^6$ cells of a 1% frequency you would only yield 288,000 per hour and so your sort would take over three hours. Then as you factor in other complicating factors such as incidence aborts, slower sheath pressure choices, etc. the time gets longer.

- How pure will my post sort sample be?
  - The more discernable your desired population in your presort sample, the higher purity you will get back. If you are trying to sort a population that is barely discernable from another population, your purity will be much lower. You also have some control in sort settings depending on what is more important to you – purity or yield. If yield is more important than purity, the incident aborts discussed above can be minimized by allowing desired events close to non-desired events to be sorted. This runs the risk of getting a few more non-desired events in your sort, but is a trade off. We recommend sacrificing a few sorted cells at the end of the sort to do a purity check.