Guide to Getting a Great Sort:

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Updated 10-28-2016

What to bring to the sort:
-your cells (on ice? If necessary, then do so! We can chill/warm your cells as they are sorted AND collected).
-suspend your cells (if possible) @ 10e8/mL in an appropriate buffer (see buffer selection below).
-single-stained controls for the colors you wish to use (good controls are an essential part of doing "good science", and those rules don't change once you enter the sorter lab!).
-a negative control would be nice
-a 15mL tube of your sort buffer
-a 15mL tube of your culture media

Make sure your cells are prepared correctly for sorting:

-suspend your cells at 10e8/mL if possible.

Why?: It is easier to dilute rather than to concentrate by centrifugation/resuspension. Cells too heavily concentrated will cause a higher than acceptable abort rate on the sorter. Cells too lightly concentrated will require longer sort times and higher pressure settings on the sorter to obtain a reasonable sort rate.

-See our webpage for sort buffer selection:

https://docs.google.com/viewer?url=http%3A%2F%2Fwww.cores.emory.edu%2Ffcc%2Fdocuments%2Fbuffer criteria7.pdf

-If your cells are adherent, split them the night before the sort.

Why?: They will dissociate much more easily the day of the sort.

-If you use a trypsin/EDTA buffer for dissociation, quench the dissociation buffer with cation-free FBS buffer.

Why?: Typically, the trypsin (or other detachment buffer) is quenched with culture media or a PBS/FBS buffer. This is problematic because it reintroduces the cations that facilitate the cells reattaching to the

plate (or each other). One must use a cation-free FBS buffer in order to stop the detachment. Additionally, the level of EDTA can be increased if necessary (but too much EDTA can be deleterious).

-**Accutase** can be used in lieu of Trypsin/EDTA (highly recommended!):
http://www.accutase.com/accutase.html

-Check your cells with trypan blue to determine cell health PRIOR TO THE SORT.

Why?: If there is anything the sorter doesn't like, it is **dying cells**. They are simply too difficult for the sorter to sort (think of it this way: it's like trying to sort peas mixed with jello…the viscosity of the media in which the cells are suspended, because of dying cells releasing their contents into that media, is not conducive to allowing the sorter to see individual events (or cells)). If that is the case, the sorter "pretends to sort", and all indications are that it is, in fact, sorting…until we look at the post-sort product…then we see low purities and low (really low!) viability. Look to our website for a link to sort data graphically displaying these kinds of results.

-If your population of interest is very rare, the sorter has to work harder in order to obtain any appreciable quantity/purity of material. It is, after all, restricted by the laws of physics (isn't everyone?). From Howard Shapiro's book "Practical Flow Cytometry". 3rd Edition. Howard M Shapiro.
(Alan R Liss, Inc. ISBN 0-471-30376-3), pg 267:
"The practical significance of all this is that two-pass sorting, with coincidences being neglected in the first pass, is virtually mandatory to improve recovery of cells that comprise a very small fraction of the total population being sorted. It is also advisable when working with rare cell types to use any bulk cell separation methods, e.g., centrifugal elutriation or immunomagnetic separation, that can be applied to increase the fraction of desired cells before undertaking flow sorting for final purification. McCoy

et al point out that triggering on fluorescence instead of scatter also facilitate sorting rare cells."

What This Means: A two-pass sort is defined as sorting your sample twice. The first sort is done with the instrument set to "enrich" your population of interest, thus the purity of the resulting post-sort sample is usually between 30 and 50%. However, since the sorter was set to enrich and not purify, it will attempt to sort ***every event that meets your sort criteria even if a contaminating event will be sorted as well!***

The first-step sort can be a high-speed sort (and often is), whereby the sorter is set to trigger on fluorescence (rather than scatter). The fluorescence trigger allows us to ignore unwanted events and to sort at a very high throughput (close to 100 million cells /hour).

The second-step sort is done with high-purity and high-recovery settings, and is more typical of how we usually run the sorter. Trigger on scatter, and since we have enriched our target population, the sorter has an easier time of it.

See our webpage for an example of a two-pass sort done in our facility:

http://www.cores.emory.edu/fcc/resources/index.html