CELL SORTING Q and A

Q 1: When should I use fluorescence activated cell sorting over bulk separation methods like panning or magnetic bead separations?

A: a) When very high purity (95%-100%) of the target population is required.

b) For separation of populations that have a low density of receptors on their surface.

c) For enrichment of populations on the basis of surface receptor density.

d) For separations on the basis of multicolor staining.

e) For separations on the basis of internal staining e.g. of DNA or of internal antigens.

Q 2: Will my cells be harmed by the sorting process?

A: Generally, cells are not harmed through the process itself as long as they are maintained continually at a temperature, pH, and in media that is most suited to them. Sort stress on fragile cell types can be reduced by using larger-sized nozzles, i.e., a 100 micron nozzle, which is operated with lower pressure than the smaller 70 and 85 micron nozzles.

Q 3: How many cells do I need to prepare to recover 1 X 10^6 of a population that comprises 10% of the cells?

A: 1 X 10^6 = 10 % target population x 50% recovery x 20 X 106 starting cell number. 50% recovery is low and routinely exceeds that number; the actual percentages of cells that are recovered from a given sort depend on a multitude of factors:

a) Cell death that occurs pre- and post sort and loss through adherence of cells to tube walls.

b) Sort rate; the higher the sort rate the lower the recovery.

c) Precision of sort set up.

d) If it is an enrichment sort or a purity sort. Enrichment sorts have higher recovery than purity sorts.

Q 4: Are there ways to improve sort recovery?

A: Yes.

a) Use polypropylene tubes for cell processing and sorting.

b) Count cells immediately prior to sorting (after all washes).

c) Use a sort precision setting of 0,16, 0 to improve yield

d) Use polypropylene collection tubes and fill them > 1/3 with media that contains 20% serum.

e) Find optimal temperature for sort (4°C, 15°C, RT).

f) Invert tubes every hour during the sort.

g) Process collection tubes immediately after they are filled up or the sort is finished.

h) Spin collection tubes ~ 10 min.

Q 5: Do I have to use the monoclonal antibodies for staining of the surface antigens at the same cell to reagent ratio as for small cell numbers?

A: Our experience suggests that incubating 10 to 30 million cells in a volume of 0.5 ml of buffer with a quarter to a fifth the amount of antibody that one would calculate as the correct amount through scaling up is sufficient for adequate staining.

Q 6: How long does an average sort take?

A: The average sort takes about 1 hour of set up time for the instrument, up to 15 minutes for setting sort regions for the cells, and about 15 minutes of post-sort analysis. At maximum speed with a 70 micron nozzle at 70 psi, sorting of 30 X 10^6 cells takes about 1 hour. Using larger nozzle sizes such as 85, 100, and 130 micron at lower pressure settings for large cell types or to reduce the cell stress will lengthen the sort time considerably. In addition, sort time is highly dependent on the percentage of the desired cells in the sample as well as the purity and the number of cells that the investigator requested for the post sort assay.

Q 7: Is it possible to sort for positive cells and negative cells at the same time?

A: Yes, they can be collected into a left and a right sort vial. In fact, it is possible to collect four different cell populations at the same time. It is also possible to collect one target cell population into one vial and all the remaining cells - the waste stream- into the other vial.

Q 8: What % of the theoretical number of cells can be recovered in reality and what does it depend on?

A: Generally, at least 50% of the theoretical number of cells can be expected to be recovered, most of the time the efficiency of sorting exceeds that percentage. The losses are caused by factors that have already been discussed in the answer to question 3.

Q 9: How do I assure cell viability during the sort and are there any ways of improving it?

A: Samples should be held in a rich medium that is most suitable to them. The addition of serum proteins is advisable, but should not exceed a concentration of 2-3% because the sort stream precision is detrimentally affected by high serum content in the sample to be sorted. The collection vials should contain a high serum concentration as this concentration is diluted by sheath fluid from the sort droplets. The vials should be maintained at the optimal temperature for the cell type sorted. Processing of the sorted cells as soon as possible after the completion of the sort helps to maintain cell viability.

Q 10: Is there an optimal suspension medium for the cells and an optimal cell concentration for a sort?

A: We resuspend cells at a concentration of 10-30 X 10^6 per ml in RPMI 1640 containing 25 mM Hepes and 2% serum because this permits to run the sort sample at a low sample pressure which improves resolution of cell clusters. However, for cell preparations which tend to clump excessively, it can be advantageous to resuspend the cells at 4-5 X 10^6 per ml.

Q 11: How many cells per second can be sorted on a BD FACSAria sorter?

A: Maximum event rate on the instrument is ~15,000 events per second when using the 70 micron nozzle. For certain cell preparations lower rates such as 5000 events per second can be advisable to obtain higher purity,

Q 12: What is the maximum purity of a population that can be achieved and what does it depend on?

A: Maximum purity is 99% to 100%. 95% to 100% purity can be expected for populations that are well resolved from the unwanted cells. It is dependent on sorter stability and the way sort gates are set. These gates should always be set with the investigator present to help make decisions.

Q 13: It is possible to do a sterile sort and put the sorted cells into culture?

A: Yes, but addition of antibiotics is advisable. Setting up multiple cultures from the collection vials can be helpful. If one of the vials is contaminated the others will allow the continuation of the experiment.

Q 14: Is it possible to sort for a population that comprises less than 1% of the total or do I have to enrich for it before the sort?

A: Yes. Rare event sorts can be done but sometimes have low purity and a low yield. To improve this, cells can be pre-enriched through bulk methods or an enrichment sort.

Q 15: It is possible to exclude dead cells from the sorted population by adding a dead cell discriminating dye or will it have toxic effects on the sorted population?

A: Live cells can be sorted on the basis of dead cell exclusion with a low concentration of DNA dyes such as propidium iodide or 7-amino-actinomycin D. The toxic effects of these dyes on the live cells should be minimal because they adhere only passively to the live cells and should be removable by washes. Various amine-reactive dead cell discrimination dyes that differ in their excitation and emission profiles are also available for exclusion of non-viable cells based on fluorescence.

Q16: Is it possible to sort single cells into microtiter plates?

A: Yes, all sorters in our facility are capable of plate sorting, however the sort set-up differs from bulk sorting, requiring extra time and advance notice.

Q 17: How many parameters can be used simultaneously for sort decisions on the FACSAria sorters?

A: The maximum number of parameters currently available on the BD FACSAriaIII sorter is 14: twelve fluorescent parameters plus forward scatter and side scatter. The SORP BD FACSAriaII sorter has 12, and the last BD FACSAria has 11 parameters. Note, however, that the three sorters in the facility differ in their lasers and configurations requiring matching the instrument to the experiment and staining reagents. Please, refer to the facility web site at http://cyto.mednet.ucla.edu for this information.

Q 18: How is the purity of the sorted population determined?

A: By re-analysis of the sorted population(s) on the cell sorter or another flow cytometer.