Aria II CELL SORTING PROCEDURE

Please schedule a meeting to discuss your experiment **before** planning your sort.

Please fill out a 'sort request form' when dropping off samples. This must have your **Activity Number** on it.

Due to the length of time required to sort cells, multiple sorts or a large number of cells may require an early morning appointment.

If you bring more samples than originally planned for or if you are late, your sort will only go until the next appointment arrives.

If you need to cancel your sort, please give 24 hours notice. This allows someone else to schedule for that time slot. If you do not cancel within 2 hours of your sort, you will be charged a cancellation fee.

A 15-minute set-up fee will be included for all sorts.

You must bring proper controls. A negative or unstained control is required per cell type. Single-stained controls are necessary for multi-color sorts. Each control should be suspended at an approximate concentration of 1 million cells per 500ul (minimum).

All samples, including controls, need to be filtered before sorting. *Please ask for filters if you need them.*

Please bring collection media and extra buffer.

***Conditions that can adversely impact your cell sample prior to sorting include cell media, sheath fluid, buffering capacity and protein concentration. Low cellular viability, auto fluorescence, and cell aggregates will result in poor sorts. Identification of these factors, and taking the appropriate action to remedy the problem will improve sort purity, yield, and cell viability.
Please note: BD FACService TechNotes is available here… \\LRI-fs01\RESEARCH\FLOWCYTOMETRY\SORTING\SortingTechNotes.pdf for more tips on cell preparation and sorting.

Preparation for Sorting Cells

Single Cell Suspension

Smaller cells should be suspended at a concentration of approximately 5-10 million cells per milliliter. Larger cells need to be somewhat more dilute, 3-5 million cells per milliliter usually works well.

Adherent Cells

Require special care for sorting:

One major problem with adherent cells in flow cytometry are aggregates. It is essential that the samples be in a single-cell suspension. Not only can aggregates cause clogging of the sorter, they may also pose a much worse problem for the investigator. For example, if there is an aggregate of two or more cells containing a positively stained cell, this event may be seen by the cytometer as a positive single event. If the event is not screened out by the scatter gates or by another parameter such as propidium iodide fluorescence for live cell/dead cell discrimination, the aggregate will be sorted. Thus, any negative cells that are stuck to the single positive cell will be sorted as passenger cells and will be co-cultured with the “sorted” populations.

Appropriate Sample Buffers for Cell Sorting

Most suspension cells do poorly at high pH. Since carbonate buffers are rather weak and allow the sample pH to increase in air (where sorting takes place), we recommend that the sample buffer be either PBS additionally buffered with 25mM HEPES (at a pH appropriate for the cells), or a culture media without bicarbonate buffers (also buffered with 25mM HEPES) or phenol red. Indicators such as phenol red (used in tissue culture media) and other compounds, such as certain B vitamins, will adversely affect the cytometer’s measurement of specific cell fluorescence, and should be avoided.

Avoid indicators such as PHENOL RED

Carefully choose the appropriate sample buffer for cell sorting. See recipe below.

Basic Sorting Buffer

- 1x Phosphate Buffered Saline (Ca/Mg++ free)
- 1mM EDTA
- 25mM HEPES pH 7.0
- 1% Fetal Bovine Serum (Heat-Inactivated)

Methods of Removing Cells from a culture dish or flask

EDTA

Proprietary "Non-Enzymatic Cell Dissociation Buffers"

Trypsin

Accutase (www.innovativecelltech.com gives more information on **Accutase)**

The most common agents used to detach adherent cells in the preparation of single-cell suspensions are trypsin, EDTA and proprietary "Non-enzymatic Cell Dissociation buffers" (these buffer solutions are available from Gibco as well as from other companies). Trypsin may damage cell surface markers and is usually avoided when the cells are to be stained with immunofluorescent probes. Avoid using proteolytic enzymes.

If the cells are exposed to Ca++ (or other divalent cations) at any time after trypsin, EDTA or cell dissociation buffer, the cells are likely to become sticky and will reaggregate. Therefore, the following precautions should be taken:

- Inactivate trypsin with a trypsin inhibitor
- Do washes in "PBS minus" (divalent cation free)

If serum must be added to the cell suspension, after treatment with trypsin, the serum should be dialyzed (to remove calcium and other divalent cations which can cause aggregation of the cells).[1]

No more than 2% serum should be added.

**If the sort was completed under less than ideal conditions, eg. proper controls not provided, non-optimal dye used…, an email will be sent to your PI to inform him or her explaining the situation.*