Cell Sorting Recommendations

Cell concentration should be about 3-5 million cells/mL. It is recommended to bring at least 10 million cells for sorting. You should bring extra sorting buffer in case the sample requires dilution.

Cell Detachment/Isolation:

You can use **ACCUTASE or ACCUMAX** (Non-enzymatic cell dissociation buffers) for detaching cells, which are the best options to use. The samples can stay in either buffer throughout your entire sort. **Take note** that Accutase can alter some surface epitopes and this effect will need to be determined empirically for the epitopes being evaluated.

The two products, Accutase and Accumax, are from Innovative Cell Technologies for cell dissociation. Accutase gently detaches confluent cells from plastic ware, does not have to be neutralized like trypsin, and preserves epitopes that can be stained by flow cytometry. Accumax breaks up aggregates in suspension culture and can extend the sort time of a sample.

http://www.accutase.com/

Recommendations for trypsinized adherent cells:

Adherent cell lines can reaggregate when serum is used to inactivate trypsin. After trypsin treatment, for detaching adherent cells in the plate, **Soybean Trypsin Inhibitor** is recommended to stop trypsinization instead of using serum. When using trypsin to detach adherent cells, you can use FBS that has been dialyzed against Ca/Mg++ free PBS or increase the EDTA concentration to 5mM or higher (first make sure that your specific cell type is not sensitive to high concentrations of EDTA). Each cell type responds differently to SBTI and the ideal conditions must be determined empirically, but usually between 0.05%-0.25% SBTI in HBSS will work.

For cell line samples, it is also recommended to add 1mM EDTA.

For sticky cells:
Try increasing the concentration of EDTA to 5mM and use FBS that has been dialyzed against Ca/Mg++free PBS or 0.5% BSA.
Caution: Some cell types can be sensitive to high concentrations of EDTA.

• Basic Sorting Buffer:

- 1X Dulbecco's PBS (Ca, Mg++ Free)
- 25 mM HEPES pH 7.0
- 1% FBS or BSA (For some cell types, BSA is better than FBS.)

Addition of **HEPES** will significantly increase the buffer capacity of the original sample buffer. Buffer capacity of the common phosphate and carbonate buffers gets compromised by high

pressure within the instrument during the cell sorting procedure. Using HEPES **is important** to sure up the cell membrane. It is even good to use HEPES during staining.

Do **NOT** use sorting buffers that contain **phenol red,** as this causes an increase in background fluorescence and decreases sensitivity. If using RPMI as your sorting buffer, which is not recommended, make sure there is **NO** phenol red (highly autofluorescent) and that 25mM of **HEPES** is added.

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.

For samples with a high percentage of dead cells:

Cellular clumping can be caused by DNA released from dead cells. If you have extensive cell death in the sample, add DNAse (deoxyribonuclease). It will not only decrease clump formation in the sample, but also will decrease viscosity of the single cell suspension buffer, by digestion of the free DNA. Sort purity and yield could go substantially up after DNAase treatment.

DNAseI in the presence of magnesium chloride will help to reduce cellular aggregation.

a) Treat cells for 15-30mins in a solution of 100ug/ml DNAse & 5mM MgCl2 in HBSS at room temperature
b) Wash cells once in HBSS with 5mM MgCl2
c) Gently resuspend cells in HBSS with 5mM MgCl2 & add 25-50ug/ml DNAse (as a maintenance dose) prior to & during the sort.
Note: DNAse requires at least 1mM Mg to work effectively, although 5mM is optimal. Actin released from dead cells irreversibly inhibits DNAse I so it is important to minimize the presence of dead cells. (EDTA should be avoided for the DNase to work properly!)

Sticky cells Cell Sorting require cells in a single cell suspension. If the cells are clumped they cause several problems such as:

• A large clump will clog the cell sorter nozzle tip 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.

Dead Cell Exclusion:

Propidium Iodide, DAPI, or 7AAD can be added for excluding dead cells during sorting. The use of these markers eliminates sorting or analyzing non-specifically stained events as wells as dead cells that can give misleading total cell yield of a sorted population and analyzed data.

Collection Media:

You should bring collection media, that range from PBS to enriched tissue culture media with antibiotics or 100% FBS to suit your cells. Using **100% serum** is recommended for collection if it works well for your protocol.

The sorted cells will be diluted with the sheath fluid.

More Tips:

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 prevent this.

Counting cells just prior to final resuspension will aid in accurately determining how many cells you are starting with, what volume to resuspend in, and estimate the maximum yield for each sorted population.

We will filter all samples using sterile 40micron filter before sorting

Sorting is not 100% efficient! There is cell loss **before, during, and after** the sorting process. The healthier the cells at the beginning, the better the results of the sort will be.

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