Buffers and Sample Preparation for Cell Sorting

Prepare the following buffer in which to suspend cellular samples prior to cell sorting.

Basic Sorting Buffer

1 x Phosphate Buffered Saline (PBS) or Hanks Balanced Salt Solution (HBSS) (Ca^{2+}/ Mg^{2+} Free.)
1mM EDTA
25 mM HEPES pH7.0
1% Fetal Calf Serum (Heat inactivated) or 1% Albumin

- Filter sterilize using a 0.2 µM filter
- Store at 4 degrees.

Cell Type Specific Buffer Modifications:

1) **Clean Lymphocyte Populations**: The buffer can be simplified to just HBSS with 1% FBS.

2) **Sticky Cells**: Raise the concentration of EDTA to 5mM and use 1% BSA instead of FBS.
   - EDTA helps prevent cation dependent cell-cell interactions

3) **Adherent Cells**: Trypsin is usually used to detach cells from the plate surface and is neutralized with media containing FBS. The FBS re-introduces cations that aids in attachment to plastic and can cause cells to re-aggregate before sorting.

   a. Use 5 mM EDTA or higher *NOTE: too much EDTA can kill your cells*
   b. Accutase and Accumax are cell dissociation products sold by Innovative Technologies that can aid in maintaining single cell suspensions.

4) **Samples with a High Dead Cell concentration**: Dead cells can release their DNA into sorting media which in turn can cause cells to clump together. Adding DNAse I in the presence of $MgCl_2$ will help reduce the aggregation.

   a. Treat cells for 15-30 minutes in a sterile solution of 100 µg/mL DNAse and 5 mM $MgCl_2$ in HBSS at room temp.
   b. Wash the cells 1x in HBSS containing 5mM $MgCl_2$.
   c. Re-suspend the cells in HBSS containing 25-50 µg/mL DNAse, plus at least 1mM $MgCl_2$ prior to and during the sort. (5mM $MgCl_2$ is optimal)

Sample Concentration:

It is important to count the cells. Sorting speeds are limited by concentration and volume. Please refer to the instruments specifications page to determine which concentration is required for your sort.

Sample Filtration:

To prevent clogging, samples MUST be filtered prior to instrument loading.

Pass the samples through nylon mesh with a pore size of 40 µM to eliminate large aggregates

- BD Falcon, 5mL Tubes with 40 µM filter top cap P/N: 352235)

Sample Collection:

The size of collection tube required depends on the amount cells you expect to retrieve.

- 15 mL conical tubes for large recoveries (one-way or two-way sorts only)
- 5 mL tubes for smaller volumes or 4-way sorts.
- Eppendorf tubes
- Multi-well Plates (96, 48, 24, 12, 6)
- Slides (Chamber, frosted)

Add media to the collection tubes to prevent the cells from drying out and dying.

- Use a concentrated media or have a high FBS content (~50%) to help cells recover from the sorting process.
- 2 – 3 mL for 15 mL tubes
- 750 µL - 1 mL for 5 mL tubes

References:

BD FACService TECHNOTES, Customer Focused Solutions, Special Sorting Issue, Vol.9 No.4, October 2004, BD Biosciences.