Useful Tips for Cell Sorting

Sample Preparation

1. Prepare your cells in media such as PBS with FBS (1-5%) or BSA (0.1-1%) to maintain viability. Use the lowest sufficient concentration for better data quality.

2. Prepare cells at the concentration of 1 to 10 million/ml. Too high a concentration results in clogging of cytometer while too low a concentration means prolonged idle time, which decreases cell viability.

3. It is essential to achieve a single cell suspension for sorting. Addition of EDTA (0.5%) may prevent cell aggregation

4. Addition of DNase (25-50ug/ml) together with magnesium chloride (1-5mM) to samples with low cell viability. This may prevent cell aggregation due to the release of DNA from dead cells.

5. Filter samples with cell strainers right before cell sorting to remove remaining clumps. BD Falcon cell strainers are available at 40µm (REF 352340), 70µm (REF 352350) and 100µm (REF 352360). An alternative is BD Falcon 5ml polystyrene round-bottom tubes with cell-strainer cap (REF 352235).

6. It is recommended that a viability dye is used to exclude dead cells.

   - For non-fixed cells, use PI, 7AAD, DAPI, SYTOX Blue (http://www.lifetechnologies.com/order/catalog/product/S11348), Green, or Red, etc.
   - For fixed cells, use LIVE/DEAD Fixable Blue (http://www.lifetechnologies.com/order/catalog/product/L23105), Aqua, Violet, Green, or Red, etc.

7. When doing multi-color staining, choose fluorochromes whose peaks are far apart on their emission spectra to minimize spectral overlap.

8. Reserve the brightest fluorochromes for dim antigens, and vice versa, to obtain the best result.

Sorting

1. While sorting large or adherent cells, keep the threshold rate lower than 3000 events per second to prevent clogging. For small suspension cells, gradually increase the sorting speed to not higher than 5000 events per second.

2. During the sorting process, pay close attention to stream stability and drop 1 position, the remaining level of sample tube, and the level of collection tubes.

3. When sorting rare cell populations, it is recommended to enrich them through bulk methods (e.g. magnetic bead separations) before sorting. Or you may do an enrichment sort followed by a purity sort to achieve the best recovery and purity.

4. When the volume of the sample is large, separate it into several tubes and sort them one by one. In doing so, you reduce idle time for each tube and prevent aggregation of cells at the bottom.

Sample Collection

1. Polypropylene tubes (REF 352002) are better for collection as they show less cell adhesion than polystyrene ones (REF 352008).

2. Fill the collection tubes with over 1/3 volume of medium with at least double concentration of serum since collection medium will be diluted by sheath fluid.

3. Rinse the tube walls with collection medium to improve cell recovery.

4. Keep the collection tubes at the optimal temperature and process the cells as soon as possible after the sort to maintain cell viability.

Useful links

http://www.bdbiosciences.com/colors
http://www.iiirflow.biology.ed.ac.uk/iiir_flow_facsaria_sorting_tips.html
http://www.rockefeller.edu/fcrc/tips/sampleprep
http://cyto.mednet.ucla.edu/Protocols/question.htm