1. Single Cells Suspension -- Ensure that samples are in a single cell suspension. **Always use a microscope** during sample preparation to track status of cells. Observe and trouble-shoot processing steps to optimize generation of a single cell suspension.

2. Controls -- **Prepare appropriate controls**, this includes:

A. **Unstained** or unlabeled control (mock transfected with vector not expressing fluorescent protein), cells not labeled with any conjugated antibody, stain and not expressing any florescent protein's.

B. **Single color controls**, one for each fluorochrome, dyes or fluorescent protein used in your experiment

C. **Note:** be careful not to add live dead stain/dye to your unstained and single color controls.

3. Filter -- **All samples must be passed through a 40um cell strainer**; we recommend BD Falcon Cell-Strainer Cap tubes (ref number 352235) as the last processing step prior to coming to your appointment.

4. Tubes -- Sort samples can be brought in either 5ml FACS tubes or 15ml conical bottom tubes.

5. Ice -- Cells should be kept on ice to prevent aggregation.

6. Concentration -- Sort samples should be re-suspended to a concentration of 20 million per ml or a minimum of 300µl.

Sort Buffer

	Component	Purpose
Basic buffer	PBS (1x) Ca++/Mg++ free	Isotonic buffer
	2% FBS or BSA	Protein stabilizer
add for pH sensitive cells	25mM HEPES pH7.0	Buffers pH in atmospheric conditions
add for adherent cells	2mM EDTA	Reduces cation mediated cell adhesion
add for dead cells, viscos samples	DNAse1	10Uml or 1ug/ml (see below for more information)

DNA released from dead cells causes cells to stick together. Minimize issues by gently harvesting cells. For sensitive/fragile cells Accumax (Sigma A7089) can be used for cell harvesting instead of trypsin (see attached product information). Accumax can also be added in the Basic buffer above (dilute 1:1 with buffer) instead of buffer alone to maintain a single cell suspension for sensitive/fragile cells.

Hint: Worthington-biochem.com is an excellent resource for products and protocols for tissue disassociation.

Collection Vessel

Sorters can accommodate the following collection vessels:

1. 5ml culture tubes
2. 15ml conical bottom tubes
3. 1.5ml Eppendorf tubes
4. Culture plates (384, 96, 48, 24, 12, and 6 well)
5. PCR plates (384 well and 96 well)
6. Glass slides, including chamber slides

Note: if you are sorting two or less populations and expect to receive greater then 1.5 million cells, please use 15ml conical bottom tubes as your collection vessel.

Coated Collection Vessels

We highly advise that you use coated tubes if you expect to obtain less then 200,000 sorted cells. It has been found that a significant number of cells can stick to uncoated tubes and drastically reduce the number of cells recovered after your sort. Please follow the simple protocol below to prepare coated tubes.

1. Use polypropylene tubes, we recommend 5ml culture tubes (BD Falcon 352063).
2. Fill tubes with PBS with 20% FBS or 5% BSA.
3. Incubate 2h at RT or overnight at 4°C
4. Just before sorting empty tubes and add your choice of collection media. This procedure works best if tubes are not allowed to dry prior to receiving cells.

Collection Media

1. For cells intended for culture; growth media or growth media with high percentage of serum, FBS, PBS.
2. Cells intended for DNA or RNA analysis can be sorted directly into lysis buffer such as Trizol. It is often advisable to sort into 1.5ml Eppendorf tubes with 700ul of lysis buffer if the total number of sorted cells is expected to be less then 200,000.
3. The volume of collection media depends on your application, collection vessel and expected number of sorted cells, generally 1ml per vessel is a good starting point.

Note: cells are sorted in PBS droplets and can significantly dilute the collection media. 1ml PBS is sorted per every 400,000 cells.

Consultation

This document is a general guide for preparing for your sort. If you have any further questions or require advice regarding any aspect of flow cytometry experiment design please contact **FCCF staff at 646-962-7611**.