

Sample and Collection tube preparation for Cell Sorting

Cells should be in a single cell suspension, so filter them well. Cell aggregates are the primary cause of poor sort results. We use a doublet discrimination gate to exclude doublets from the sort gate, but it is not 100% effective and cell aggregates may result in poor purity. *It best to filter the cells, using <50-micron filter, immediately before coming to the facility (there are filters available in the flow cytometry facility if you don't have them).*

Resuspend cells at a concentration of at least 10 million cells/ml (or a minimum volume of 300ul if you don't have enough cells). Buffer of your choice; **MUST BE <1.5% SERUM OR BSA.** It is best to put the cells in a 5ml (12x75mm) tube with a cap. (Polypropylene tubes work best; sterile 5ml polypropylene are available at the Wistar Research Supply Center or Flow Cyto Facility).

You will need tubes or plates to collect the sorted cells. We can sort into 15ml, 5ml (12x75mm) tubes, or 1.5ml microfuge tubes. Droplets containing sorted cells will be at a concentration of:

- ~1 million/ml in sorting buffer (PBS), if using the 70u nozzle (PBMC, BM, spleen, leukocyte-based cell lines),
- ~440,000/ml using 85u nozzle (many cell lines),
- ~300,000/ml using 100u nozzle (**larger cell lines, tumors**).

If sorting into plates for viable cells, you will need to have some collection media in each well, usually 50 – 100ul if sorting for single cells. If you sort single cells into PCR plates, please let us know so we can discuss this further.

Collection media: For viable cells, most people sort into media with 50% serum. If viability is not as important for you, use anything.

Amount of collection media suggested:

- 3ml collection media in a 15ml tube,**
- 750ul collection media in 5ml tube,
- 100ul in 1.5 microfuge tube.

Keep in mind the concentration of cells depending on nozzle size (see above). Plan accordingly and bring a few extra tubes.

If you are collecting cells for RNA/DNA, please do not use Trizol for collection media. It causes problems.