UNIVERSITY of MARYLAND
MARLENE AND STEWART GREENEBAUM
COMPREHENSIVE CANCER CENTER

Flow Cytometry Shared Services

Sample Preparation:

The key for a fast, pure sort is to avoid aggregation. Aggregation can clog the nozzle, disturb droplet formation. The result is poor purity and slow sort.

Sample buffer: PBS + 1% BSA + 1 mM EDTA. Protein and Ca/Mg increase aggregation. Serum has both, so avoid it if cells can survive.

Use 1 mM EDTA throughout, starting from the time harvesting the cells. This improves the sort efficiency. On a PBMC sample, without EDTA, efficiency 60%; with EDTA, over 90%.

If you use trypsin to detach the cells, do it as short as possible. 3-5 min range. Longer time results in aggregation. Avoid using high concentration of FBS to neutralize. More protein covering the cells will increase aggregation. 1% FBS is good, 2% max. Centrifuge gently, with just enough speed to bring cells down.

Too many dead cells and DNA released from them can make the sample sticky.

Sample concentration: 3 million/ml for adherent cells, 10-15 million/ml for non-adherent smaller cells such as lymphocytes. Sort time: 2 ml / h. When counting the cells, please include dead cells and debris. Sorter needs to see them to gate them out.

Keep samples on ice. Filter samples using Falcon 5 ml tube with strainer cap (352235). Bring 10 ml sample buffer so I can dilute and rinse.

Collection:

Using 100um nozzle, you will get 3.5 ml of PBS / 1 million cells.
85um nozzle, 2 ml PBS/ 1 million cells.

Can sort into 1.5 ml, 5 ml, 15 ml tubes and 96 well plate.

Collection tubes can be:

BD Falcon 5 ml collection tube (Ref 352058)
BD Falcon 15 ml polystyrene conical tube (Ref 352095)

Buffer in collection tubes, PBS+10% serum. Coat 2/3 of the tube with it overnight or 1 h in advance. Just to wet the tube wall and coat it with proteins. You can use other buffer, just avoid culture medium, because PH is wrong without CO_2.