Scripps Research

Cell Sorting Guide

Flow Cytometry Core
Scripps Research - Jupiter, Florida

Sample Preparation:

The outcome of the cell sorting process will only be as good as the cell preparation. Most importantly, it is critical to minimize clumps in the sample to be sorted. Clumping is usually caused by inadequate dissociation of cells and/or cell death and release of DNA from dead cells. For these reasons, we recommend the use of a proper sort buffer containing DNAse, and passing the sample through a hypodermic needle prior to sorting. A typical sort buffer consists of:

- 1x PBS or 1x HBSS (Ca/Mg++ free)
- 1-5mM EDTA
- 25mM HEPES pH 7.0
- 1-5% FCS/FBS (Heat-Inactivated)
- 10units/mL DNase II
- 0.2um filter sterilize

Users are encouraged to filter their samples through a maximum filter size of 50 um prior to sorting. We also recommend that you bring your samples inside a syringe fitted with a 25 g needle, especially if sample volumes exceed 500 ul; passing samples through the needle prior to sorting will minimize clumping. Please note that flow blockages are detrimental not only to your sort, but to subsequent users as well. If your samples repeatedly cause problems, we will work with you to optimize your protocols, and in extreme cases (mainly involving a lack of cooperation), we may be required to deny further sorting requests until the issues can be resolved.

Instrument Controls:

- For sorting on fluorescent parameters, a negative (unstained) control as well single color controls for each parameter are required.
- For each control we recommend volumes no less than 500 ul with a least 250,000 cells/beads per control.

Sample Collection:

- 2-way sorting into 1 ml, 5 ml, and 15 ml collection devices
- 4-way sorting into 1 ml and 5 ml collection devices
- Plate sorting into 6, 12, 24, 48, 96, 384* well plates
- Slide sorting

*Sorting into a 384 well plate is possible but will require optimization for the user.
The ARIA is equipped with a recirculating water bath that allows the collection chamber to be cooled to 4° C. This feature is only available for 2-way sorting into 15 ml tubes, 4- way sorting into 5 ml tubes, and plate sorting.

Collection Tubes

It is recommended that users collect sorted cells into 12x75 mm GLASS tubes pre- coated with serum/media. Pre-coating collection tubes with serum/media will increase post-sort viability and recovery, while glass minimizes static deflection of charged sort droplets. Sorting into polystyrene and polypropylene tubes may reduce recovery as cells have a tendency to stick to plastic, and these plastics can also build up a static charge.

Although the instrument is routinely cleaned and checked for sterility, it is recommended bulk samples are thoroughly washed and antibiotics, 1x PSN and/or 50ug/ml gentamicin, are added to culture media for samples sorted for cell culture, especially primary cell culture.