# Cell sorting using spectral flow cytometry

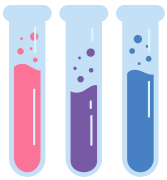
**The Emory Pediatrics/Winship Flow Cytometry Core recently acquired a new BD cell sorter, replacing the FACS Aria II. The new BD S8 imaging + cell sorter and the Cytek Aurora CS cell sorter are spectral, therefore we suggest the following strategies to ensure successful sorts:**

## Optimize cell concentration

**Final cell preparation should be as concentrated as possible**, up to 10-20 million cells per mL. If you have less than 2 million then re-suspend in 300uL of liquid. Live cells should be kept on ice as much as possible from the moment they are isolated until they are run on the machine. Once stained, cells should be kept in the dark.

## Filter the sample

Filtering the sample before sorting is essential to eliminate clumps and debris that could clog the sorter. Use a cell strainer [PN 352235, Falcon] or a nylon mesh with a pore size of no more than 50 µm to filter the sample.



## Bring appropriate controls

**Unstained cells**: An unstained cell control is required for determining cellular autofluorescence. In this way, unmixing algorithms can isolate and extract the spectral contribution of autofluorescence from the rest of the spectral signatures and improve signal resolution.

**Single stain controls:** Both cells and beads can be used and should be tested to determine which control provides the most accurate fluorophore spectral signature for unmixing.

## Use appropriate buffer solutions

Final buffers should include BSA or FBS, DNAse solution to help prevent aggregation, and HEPES buffer [Flow Cytometry Protocols To Prevent Sample Clumping]. For cells that are generally "sticky" and more likely to clump together, a small amount of EDTA can be added to help abate this problem.

Feel free to contact the core with any additional questions or for consultation purposes: ajrae@emory.edu

Emory Pediatrics/Winship Flow Cytometry Core web page

Sort request form

Assistance request form

ISAC RECOGNIZED
Shared Resource Laboratory

Guide to getting a good sort

Instrument booking web page

EMORY UNIVERSITY

Pediatrics/Winship Flow Cytometry Core

EMORY WINSHIP CANCER INSTITUTE
National Cancer Institute-Designated Comprehensive Cancer Center