Preparing Cells for Cell Sorting

How should I prepare my cells?

- Please prepare the cells in a 5ml sterile FACS tube

Aria/AriaFusion/SymphonyS6/ Melody/Discover S8	FisherScientific Cat# 352054 (Corning Falcon Round-Bottom Polystyrene Test Tubes)
Influx & All Sorters Above	FisherScientific Cat# 352063 (Corning Falcon Round-Bottom Polypropylene Test Tubes)

- Fisher Cat# 352063 should be used for adherent cell lines or whenever cell adhesion to the surface of the tube may be an issue.
- We do not recommend bringing the cells in media. Media may increase clumping of cells, causing high abort rates and clogging, which leads to poor yields.
- The cells should be suspended in *1X PBS, which is Ca++/Mg++ free, with about 1-2% BSA*. If your cells are especially sensitive, add 25mM HEPES.
- If your cells do not tolerate PBS, we also recommend using Hank's Balanced Salt Solution (HBSS) buffer, which is Ca++/Mg++ free and without Phenol Red, and contains BSA and HEPES. Suspend cells at the following concentration, with no more than 3 to 4 mL/tube.

Sort Type	Speed/Pressure	Typical Cell Type	Concentration	# of Cells Sorted per Hour
70μm Nozzle (High speed)	High/70 psi	Lymphocytes	40-50x10^6 cells/mL	70x10^6
85μm Nozzle (Discover S8 only)	Med/45 psi	Lymphocytes	20-30x10^6 cells/mL	36 x10^6
100μm Nozzle	Low/20 psi	Cell lines, tissues, neurons, dendritic cells, nuclei, CAR T-cells	5-10x10^6 cells/mL	20x10^6
130μm Nozzle (Symphony S6 and Discover S8)	Low/10 psi	Large cell types, cell lines, tissues, delicate cells	3-5x10^6 cells/mL	10x10^6
140μm Nozzle (Influx A only)	Low/5.5 psi	Large cell types, cell lines, tissues, delicate cells	3-5x10^6 cells/mL	10x10^6
200μm Nozzle (Influx A only)	Low/3.5 psi	Large cell types, cell lines, tissues, delicate cells, clusters, megakaryocytes	3-5x10^6 cells/mL	5x10^6

Special Note:
These concentrations are estimates for pristine cell preparations with no aggregates and little cell death. Platelets, RBCs, and other undesirables count as trigger events and will reduce the sorting rate. Adherent cell lines are notoriously difficult; the best preparations are usually made from cultures less than confluent and released with EDTA only. To reduce aggregation, centrifugation of all samples is best in round bottom (not conical) tubes. All samples should be passed through a 35µm or smaller mesh filter immediately before being transported to the Flow Cytometry lab. Falcon polystyrene tubes with 35µm strainer cap can be purchased from FisherScientific (Cat# 352235-Corning Falcon Round-Bottom Polystyrene Test Tubes with Strainer Snap Cap, 5mL)

- Exclusion of dead cells by the addition of nucleic acid vital dyes can improve the precision of detecting dim or rare cells. Many different dyes can be used to determine dead cells. Here is a list of the more common dyes used for live/dead exclusion:

DAPI	Fixable Live/Dead Dyes
7AAD	Draq dyes
PI	To-Pro-3

How should I collect the cells?

- Tubes: Cells can be collected in a variety of tubes. All tubes should contain some type of media to help cushion the impact when the cell lands in the tube, as well as help with cell viability and reduce sticking to the tube wall. The collection tube fluid should ideally be media with HEPES. The media should also contain antibiotics to prevent contamination. This is a list of the tubes that can be used for collection:

Type of tube	Starting collection media amount	Total # of populations that can be sorted
1.5mL (Eppendorf Tube)	300-500µL	1-2 way sorting- Melody 1-4 way sorting- Aria/AriaFusion 1-6 way sorting- Influx/Symphony S6/Discover S8
5.0mL (FACS Tube)	1.0mL	1-2 way sorting- Melody 1-4 way sorting- Aria/AriaFusion 1-6 way sorting- Influx/Symphony S6/Discover S8
15.0mL (Conical Tube)	3mL	2 way sorting only- Aria/AriaFusion/Influx/Discover S8 4 way sorting- Symphony S6

You cannot mix 5 ml and 15 ml collection tubes in a single sort. Always bring extra collection tubes and media in case of clogging.

- Micro-plates: If sorting into trays (96, 24, 12, or 6 wells), the same rules apply, except you would use the appropriate amount of media for the size well. Only 1 population can be sorted at a time into plates.
- Cells may be collected directly into RNA or DNA lysis buffer. In these cases, dilution of the lysis buffer is a prime concern. Most users are sorting up to 10^5 cells into 0.5 mL of lysis buffer in 1.5 mL Eppendorf tubes. If using Trizol as the collection buffer, use Trizol LS with a reagent to liquid sample ratio of 3:1.

Notice:

No primary human or primate samples, infectious agents, or cells transfected with non-replication deficient viruses may be sorted outside of our Biohazardous Cell Sorting Facility. The facility does not sort any radioactive samples.