Dana-Farber Cancer Institute
Cytometry Cores

DFCI Cytometry Core's BEFORE YOU SORT

DFCI Flow Core
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Planning Your Sort

Instrument Considerations

It is important to understand that different flow cytometers have different configurations. Verify that all of your chosen fluorophores can be excited and detected by our cell sorters. Refer to our laser charts, under the instruments tab on our website, or contact the core to verify.

Certain types of assays may also only be run on certain instruments.

Key Instrument Limitations:

Ratio Sort → JF Aria & M Aria ONLY

UV Fluorophores → JF Symphony UV ONLY

BV570/Pac Orange → JF Aria & M Aria ONLY

6-way Sorting → JF Symphony UV & M Symphony ONLY

Sony MA900 Sorters are Co-linear systems, larger assays may not be compatible, make sure to confirm with core

Our Cell Sorters

BD FACSymphony S6

BD FACSAria II

SONY MA900

Selecting Sort Settings

Nozzle Size

The Core offers two nozzle sizes, 70um & 100um. It is important to know which nozzle you will want to use for your sort.

70um Nozzle

- Default nozzle size
- Ideal for small—moderately sized cells
  - PBMCs, Bone Marrow, Splenocytes, Lymph Nodes
- Ideal for suspension samples
- High pressure, high throughput

100um Nozzle

- Larger Cells
  - Primary Tissue Isolation, Immortalized Cell Lines, Primary Cells Stimulation In-Vitro
- Default for plate sorting
- Lower Pressure, ideal for fragile cells

Collection Vessel

Collection tubes must be prepare for each sort. For rare populations (under 10%), Eppendorf tubes are recommended. Refer to the guidelines below.

Plates

6, 96, 384 well culture plates & 96 well PCR plates

~200-250uL of media for 96 well plate

Use 100um nozzle for plates

	1.5mL Eppendorf	5mL Tube	15mL Tube
Media to Add	500 µL	1,000 µL	5,000 µL
Capacity: 70um	1 million cells	4 million cells	10 million cells
Capacity: 100um	250,000 cells	1 million cells	3 million cells

Sample Preparation & Considerations

Buffers

Use PBS or HBSS:

Avoid buffers containing Mg or Ca, this can lead to cell clumping

FBS/BSA Inclusion:

Helps increase lifespan of cells and prevent non-specific binding

Use FBS (<2%) or BSA (<.5%)

Additives:

Aggregation preventative additives can, and should be explored as well. Discussion with the core prior is encouraged.

Aggregate Prevention

Aggregate prevention is crucial to ensure the sorter does not clog

* Use Ca/Mg free buffers
* BSA instead of FBS, remember <.5%
* Add EDTA to samples (2-5mM)
  * Consult with Core prior to adding
* Dilute samples that clump often

A sample containing a large portion of dead cells will also tend to clump more often

- Treat cells with DNAse I
  - Note: EDTA inhibits DNAse
- Use a viability dye (DAPI, LIVE/DEAD)

In certain tissue preps, it may be beneficial to use a debris-excluding dye such as Calcein. This will help with live cell identification.

If dealing with lots of aggregation, please inform the core. Switching to a larger nozzle, or filter right before acquiring samples may help prevent clogging

Controls

Controls are vital to correct set-up of a sort and should be brought for every experiment

At a minimum, it is recommended to have an unstained (or untransfected) control and a single-color control for each of your fluorophores

Single-color controls should not contain your viability dye, only a single fluorophore

Optionally, you may choose to bring FMOs
FMO = Fluorophore Minus One
i.e. A sample stained will all fluorophores in your panel except one

Cellular controls are always preferred over bead controls

On Sort Day

Final Sample Preparation

* Samples brought in 5mL FACS or 15mL conical
* Optimal concentration is 10 million cells / mL
* Filter samples 15 minutes prior to sorting

Where To Go?

The sorting facility is located in Mayer 307.

If you are going to be late, please contact the Core.

Frequently Asked Questions

Q: How long do I need to book?
A: Typically, sorting rates average around 20 million cells per hour. Please consult with core staff when scheduling your sort

Q: How pure will my population be?
A: Population purity depends on factors like sorting speed, gate parameters, cell characteristics, and adherence. The core will perform a purity check if a sufficient number of cells are sorted