Job Aid

BD FACSDiscover™ S8 Cell Sorter: Sorting into tubes

This job aid contains instructions for how to sort into tubes and analyze the post-sort data in BD FACSChorus™ Software. For additional information, see the *BD FACSDiscover™ S8 Cell Sorter with BD CellView™ and BD SpectralFX™ Technology user's guide*.

Start up system → Set up experiment → Establish settings → **Sort and analyze** → Manage data → Shut down system

Before you begin

- Start up the system and run the daily fluidics startup procedure.
- Add and design an experiment, adjust your scatter and spectral gains and Region of Analysis (ROA) for your sample.
- Perform spectral unmixing by recording data for single-stained controls, if applicable.
- Record pre-sort data and create sort gates on the View Data page.

Working with the Set Up Sort tab

Preparing the sort

1. Click the **Set Up Sort** tab.

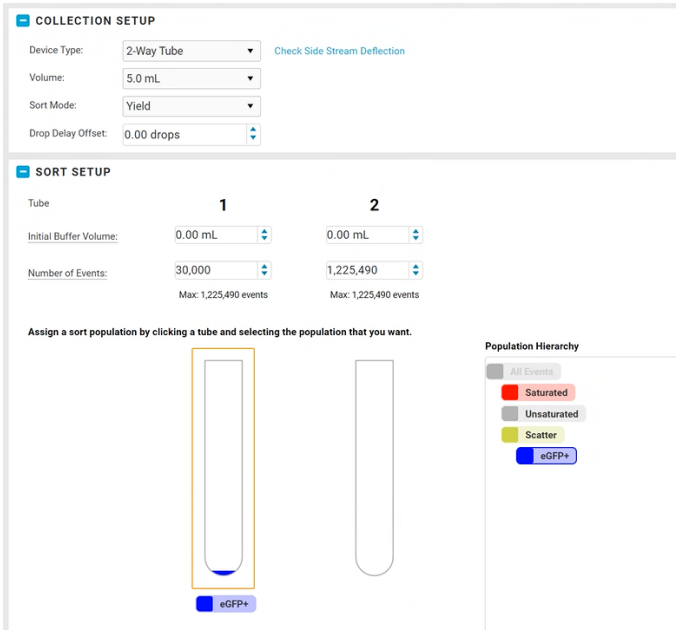
2. Make appropriate selections in the Collection Setup panel.

3. In the Sort Setup panel, enter the starting buffer volume for each collection tube.

4. Assign populations to tubes by selecting a tube, then clicking the population of interest in the population hierarchy.

   NOTE A population does not need to be assigned for every tube.

5. Assign the target event count for each tube.

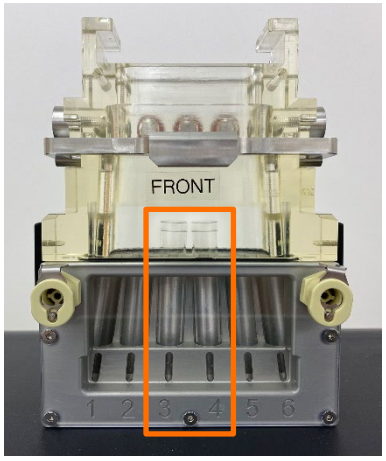


Loading the collection device

1. Insert your collection tubes into the collection device.

   Load tubes from the inside out. For example, a 2-way sort will use slots 3 and 4 in the collection device. A 4-way sort will use slots 2 through 5.

2. Install the collection device onto the bottom of the sort block.



Working with the Sort tab

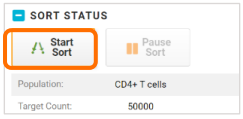
Sorting

1. Click the **Sort** tab.

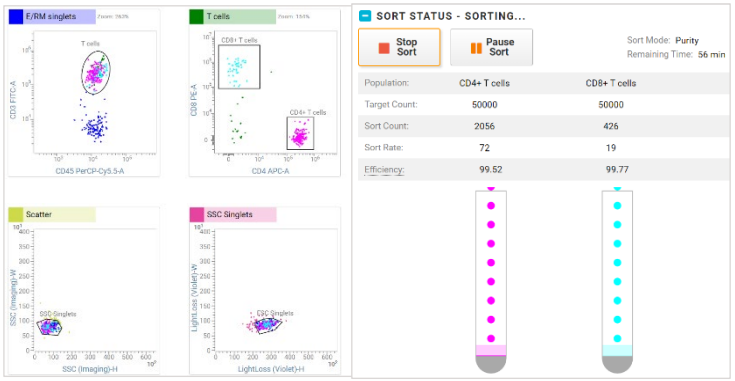
2. Load the sample tube and adjust the flow rate, if needed.

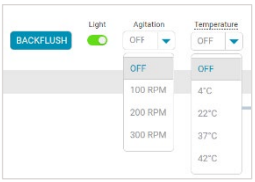
3. Click **Start Sort** in the Sort Status panel.

   NOTE The instrument will take several seconds to initialize the sort before the sort begins.

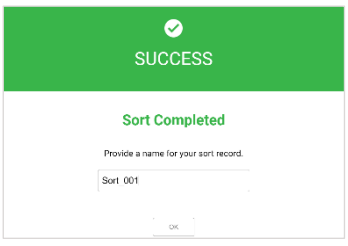


4. Monitor the sort as it progresses.
   - Adjust gates as needed in the Sort Population Plots and Additional Plots panels.
   - Monitor the sort count and efficiency of your sorted populations in the Sort Status panel.
   - Record additional data while the sort progresses, if needed.
   - Adjust the sample tube's temperature and agitation speed in the dashboard.
   - Toggle the light switch to help visually monitor the sample volume as the sort progresses.





5. When the sort finishes or is stopped, name the sort report.



6. Unload the tube, if needed.

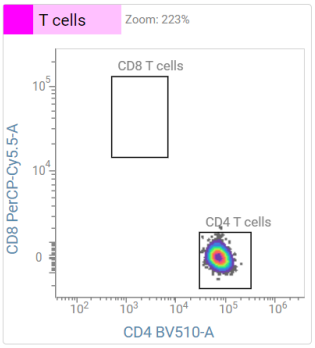
(Optional) Checking post-sort purity

1. Click the **View Data** tab.

2. Set the FCS Stopping Criteria to 1,000 events. Toggle on or off the Images Stored switch.

3. Record the collection tubes.
   a. Click **Backflush** in the dashboard. Click **OK** to clear the dialog.
   b. Load a collection tube.
   c. Click **Record**.
   d. Name the post-sort data file.
   e. Repeat steps a through d for each collection tube.

4. View the Statistics panel to verify post-sort purity.



STATISTICS

Population	Events	% Parent	% Total
All Events	1,000	N/A	100.00 %
Saturated	0	0.00 %	0.00 %
Unsaturated	1,000	100.00 %	100.00 %
Scatter	989	98.90 %	98.90 %
SSC Singlets	986	99.70 %	98.60 %
FSC Singlets	972	98.58 %	97.20 %
Singlets	970	99.79 %	97.00 %
CD45+	970	100.00 %	97.00 %
T cells	970	100.00 %	97.00 %
CD4 T cells	968	99.79 %	96.80 %

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