

# Job Aid

## BD FACSAria™ Platform

### Performing Index Sorting

This job aid contains instructions for performing index sorting using BD FACSDiva™ software. Review the entire job aid prior to index sorting. Review the Tips and Considerations When Index Sorting section for additional information and background.

#### Before You Begin

Setting up for index sorting uses the same steps as required in setting up for a plate or slide sort.

Review the instructions in these sections of your cytometer's user guide to ensure that the sort settings and hardware are set up appropriately:

- Setting Up for Sorting
- Setting Up for Sorting Onto a Plate or Slide

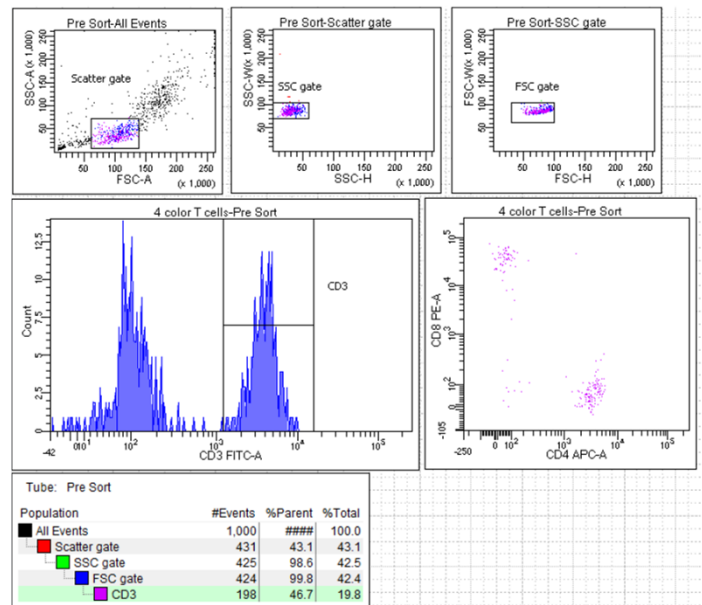
#### Setting Up the Experiment

- ① Create a new experiment for the index sort.
- ② Use a global worksheet to create the plots and gates needed to define the populations of interest.
- ③ Collect a pre-sort data file to determine the approximate event rate for your population of interest.

**CAUTION:** If the sort population drops below two events per second, the sorted event may fail to index.

**NOTE:** If you are sorting a rare population, record a large enough pre-sort data file to accurately estimate the required event rate to achieve at least two sort population events per second.

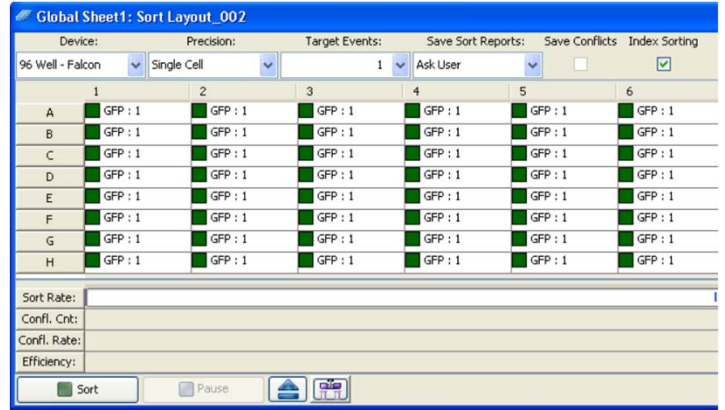
- ④ Create a new sort layout or select an existing sort layout defined for index sorting.




⑤ Make the following selections in the **Sort Layout** window.

- Precision = Single Cell (recommended setting for most applications)
- Target Events = 1
- Index Sorting checkbox = Selected


Make the other selections in the **Sort Layout** window according to your experiment requirements.



## Starting and Monitoring the Sort

- ① In the **Sort Layout** window, click the **Access Stage** button (  ) to bring the plate loader to the front of the sort chamber.
- ② Open the sort collection chamber door and install the plate or slide.
- ③ Click the **Sort** button in the **Sort Layout** window to start the sort.  
You must be acquiring sample to start a sort.
- ④ Monitor the event rate and sort population statistics to ensure that at least two sort population events per second are available to the system. Increase the event rate as needed.

**CAUTION:** If the sort population drops below two events per second, the sorted event may fail to index.

When the index is complete, a new plate analysis (  **INX\_Sort\_001** ) is added to the experiment.

The plate analysis contains the index sort data.

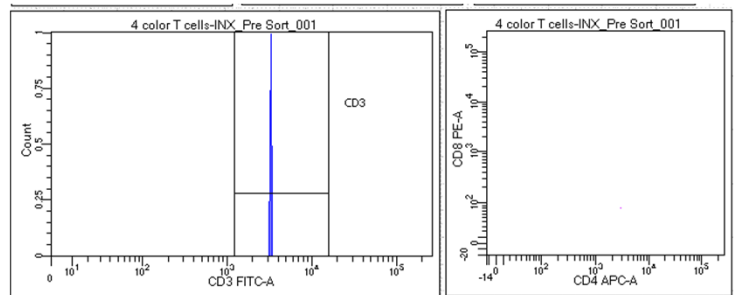
## Index Sorting Analysis

- ① When the sort is finished, unload the sort tube and carefully remove the plate or slide from the sort chamber.
- ② Right-click the plate analysis in the experiment and select **Index Sorting Analysis**.

**NOTE:** To confirm that the index sorting is proceeding without error, you should always view the **Index Sorting Analysis** immediately after sorting.

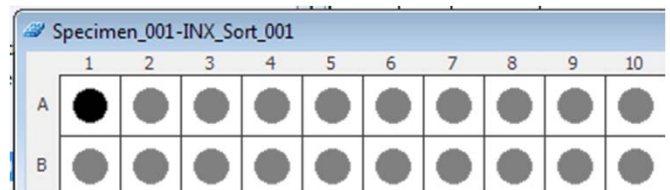
- ③ Select a well in the **Index Sort Analysis** to see the event sorted into that well displayed in a plot.
- ④ To see the statistics for an index sort, right-click a plot and select **Create Statistics View**.
- ⑤ To export the statistics for an index sort as a CSV file, right-click in the statistics view and select **Export Index Sort Statistics**.

In the dialog that opens, navigate to the location where you want to save the CSV file and click **Save**.



Tube: INX\_Pre Sort\_001

Population	#Events	%Parent	%Total
All Events	1	####	100.0
Scatter gate	1	100.0	100.0
SSC gate	1	100.0	100.0
FSC gate	1	100.0	100.0
CD3	1	100.0	100.0



# Tips and Considerations when Index Sorting

## Preparing for Index Sorting

- Prior to performing an index sort that involves multiple plates, restart the software to clear the heap memory and improve performance.
- Any size nozzle might be used for index sorting. For index sorting of a rare population, a smaller nozzle size might be appropriate to maintain a sort event rate of two sort events per second.
- A doublet discrimination gating strategy should be used when sorting. Use the Doublet Discrimination Gating analysis template provided or customize your own gating strategy.
- To determine an appropriate event rate for the target population, use the pre-sort data statistics to estimate the minimum rate needed to achieve at least two sort population events per second. Calculate the event rate needed by using the following formula:

$$\frac{(2 \times \# \text{ events for All Events})}{\# \text{ events for target population}} = \text{minimum event rate}$$

For example, if you collect 10,000 events and the target population is 10 events, you will need your event rate to be 2,000 events per second.

Tube: Pre-sort			
Population	#Events	%Parent	%Total
All Events	10,000	###	100.0
P1	1,613	16.1	16.1
P2	10	0.6	0.1

$$\frac{(2 \times 10,000 \text{ events for All Events})}{10 \text{ events for target population}} = 2,000 \text{ minimum event rate}$$

- Any precision mode is available for index sorting, but the single cell precision mode is recommended for most applications. However, for index sorting of a rare population, the purity precision mode might be appropriate to maintain a sort event rate of two sort population events per second.
- Do not change the precision mode for the plate once the sort has started.

## Plate Analysis File

The plate analysis is a special file that is specifically linked to a plate's indexed data.  INX\_Sort\_001

- Do not use the **Duplicate without Data** command or create templates on any item that contains a plate analysis. Doing so reverts the analysis to a tube, which is unusable.
- To archive the data, export the entire experiment. Do not export the plate analysis file as an FCS file because doing so will lose the indexed data. (Only FlowJo version 10.2 and higher can read exported plate analysis files.)



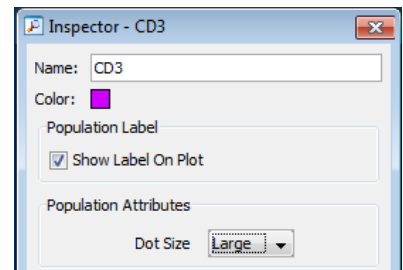
# Tips and Considerations when Index Sorting, Continued

## Analyzing Index Sort Data

- When you select a well in the **Index Sort Analysis**, the selected well's color will indicate the well status:

Color	Indication
Black	Sorted well data is showing in the plots
Gray	Sorted well data is available, but not showing in the plots
White	No sorted events
Red	More than one event sorted into a well

- To increase the size of a dot on a plot for better visibility, open the population hierarchy, select a population, navigate to the Population Inspector and select a larger size.



- FlowJo v10.2 and newer allows for offline analysis using the BD Index Sorting plug-in. You can download the plug-in from [flowjo.com/exchange](http://flowjo.com/exchange).

To analyze in FlowJo:

- In BD FACSDiva software, right-click the plate analysis file and choose **Export > FCS files**.
- Import the FCS files into FlowJo and run the plug-in to automatically generate derived parameters corresponding to the axes of the plate or slide.
- Drag and drop the plug-in node to the **Layout Editor** window to display an illustration of the plate.

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BD Life Sciences, San Jose, CA, 95131, USA

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