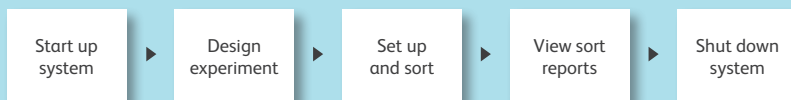


BD FACSMelody™ Cell Sorter Quick Reference Guide

This guide contains instructions for using the BD FACSMelody™ cell sorter with BD FACSCorus™ software version 1.1 and later.

Workflow Overview

The following figure shows the daily cell sorter workflow when using the BD FACSMelody system.



Start up system

Check fluids

- 1 Fill the sheath tank with sterile 1X phosphate-buffered saline (PBS).
- 2 Empty the waste tank and add approximately 1 L of undiluted bleach or a sufficient amount so that 10% of the total volume is bleach.

Fluidics startup

- 1 Press the power button on the front of the cell sorter unit.
- 2 Start BD FACSCorus™ software by clicking the shortcut on the desktop and log in. The software has been designed with guided, simple, task-oriented screens. There are numbered tabs across the top of the workspace to indicate the order or workflow where information needs to be added.

1 Fluidics Startup 2 Cleaning 3 Sort Nozzle 4 Cytometer Setup (CS&T) 5 Drop Delay

- 3 Once the system has connected, click **Run Daily Fluidics Startup**.

The screenshot shows the BD FACSCorus software interface with the following status information:

Cytometer Connection	Sheath Tank	Waste Tank
✓ Connected	✓ 13 Hr 40 Min remaining	✓ OK

Below the status information, the following text is displayed:

Last Shutdown: 06/10/2016 1:21 PM Type: Daily
Last Fluidics Startup: 06/10/2016 12:25 PM Type: Daily

At the bottom, there are three buttons: **Run Daily Fluidics Startup** (highlighted with a red box), **Run Extended Fluidics Startup**, and **Skip**.

- 4 Follow the prompts on the screen for each numbered step.
- 5 After fluidics startup is complete, click **Continue** to see the cleaning options.

1 Fluidics Startup 2 Cleaning 3 Sort Nozzle 4 Cytometer Setup (CS&T) 5 Drop Delay



Cleaning

Performing a flow cell clean is recommended at the end of the day. It is an optional step before sorting.

- 1 Click **Flow Cell Clean** or **Skip**. If you are performing an aseptic sort, click **Prepare for Aseptic Sort**.
- 2 Follow the prompts for each numbered step of the cleaning procedure.
- 3 After cleaning is complete, click **Continue** to insert the sort nozzle.

Select the cleaning that you want to run.

Prepare for Aseptic Sort

Cleans the sheath and sample paths with bleach, DI water, and ethanol.

Last Run: 06/15/2016 12:21 PM

Flow Cell Clean

Cleans the sample path and fills the flow cell with DI water. Run this procedure when poor optical performance indicates that additional cleaning is needed.

Last Run: 06/15/2016 12:22 PM

Skip

Sort nozzle

1 Fluidics Startup 2 Cleaning 3 Sort Nozzle 4 Cytometer Setup (CS&T) 5 Drop Delay

- 1 Insert the sort nozzle straight into the bottom of the flow cell cuvette with “TOP” facing up. Turn the nozzle-locking lever clockwise to the 12:00 position, and click **Continue**.

Instrument and sort quality control

We recommend running Cytometer Setup and Drop Delay daily before performing any experiments.

1 Fluidics Startup 2 Cleaning 3 Sort Nozzle 4 Cytometer Setup (CS&T) 5 Drop Delay

- 1 Prepare a tube of BD™ CS&T RUO beads by following the package directions for the BD FACSMelody cell sorter.

Run Cytometer Setup daily before you perform any experiments.

Last Cytometer Setup Run: 02/21/2017 10:59 AM
Status: Passed
To view reports, on the Cytometer Page, select Cytometer Setup Reports.

Verify the optical configuration.
Change if needed.

Optical Configuration

View or change optical filters to ensure that they match the fluorochrome emissions in your experiment.

Current configurations:
YellowGreen-4 Blue-2 Red-2

Bead Lot File

Change the bead lot file for CS&T.

Lot Number: 6284594
Expiration Date: 08/31/2017

Verify the bead lot number and expiration date.
Change if needed.

Run Cytometer Setup Skip

- 2 Click **Run Cytometer Setup**.
- 3 Load the tube and follow the prompts.
- 4 After CS&T has completed successfully, prepare the BD FACS™ Accudrop beads according to the package directions and click **Continue** to run Drop Delay.
- 5 Load the tube and follow the prompts.

Experiments

- 1
- Click **New Experiment** and provide the experiment's information. You can also select and duplicate an existing experiment from the experiment list.

- 1 Design Experiment
- 2 View Data
- 3 Set Up Sort
- 4 Sort
- 5 View Reports

EXPERIMENT INFORMATION

Experiment Name:

TREG

☐ Use as Experiment Template

Description:

TREG Experiment

Sample Temperature:

Off

Name the experiment, give it a description, and select the sample temperature. Select the **Use as Experiment Template** option if you want to make additional experiments based on this experiment.

FLUOROCHROMES & LABELS

Fluorochromes

+

PE-Cy7

+

PerCP

PerCP-Cy5-5

PerCP*

+

PE

PE*

+

FITC

BB515

+

BV510

V500

+

BV421

V450

+

APC-Cy7

APC-H7

+

APC

Alexa 647

Labels

CD4

CD25

CD127

Select from the listed fluorochromes, or click the plus sign (+) to add a new fluorochrome.

Optional: manually enter the label information for each fluorochrome in the experiment.

Tool Tip: hover over the plus sign (+) or any of the colored rectangles for laser and filter information.

3

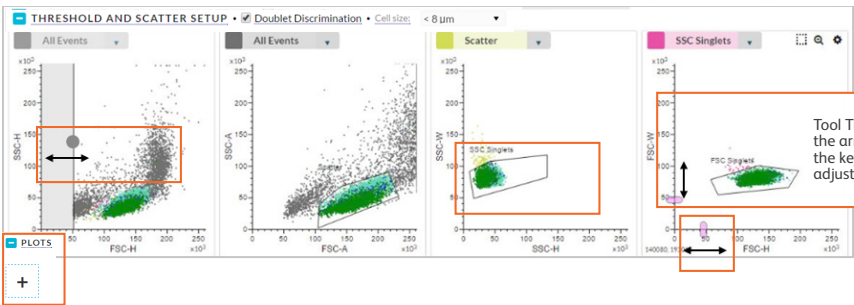
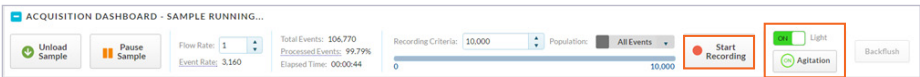
View data

Optimize the threshold and scatter setup, then collect a pre-sort data file.

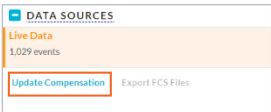
- 1 Click the **View Data** tab.



- 2 On the Acquisition dashboard, click **Load Sample** and adjust the flow rate as needed.
Optional: turn on the sample injection chamber light and agitation option.
- 3 Select the cell size and use the sliders along the plot axis to adjust the live data cytometer threshold and PMT voltage.
- 4 Adjust the gates on any plot as needed and select the population to display in the plot. Click **Plots (+)** to create additional plots as needed to define your population(s) of interest.



- 5 **Optional:** If you are running your own compensation controls, click **Update Compensation** and follow the guided prompts. Otherwise, the default system compensation values will be used.



- 6 Select the Recording Criteria and click **Start Recording** on the Acquisition dashboard to collect a pre-sort data file.

Set up sort

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



COLLECTION SETUP	
Format:	<div>Tube</div>
Volume:	<div>5.0 mL</div>
Sort Mode:	<div>Purity</div>

From each pull-down menu:
Select the format of the collection device: Tube, Plate, or Slide.
Select the volume of the sort device.
Select the sort precision mode: Yield, Purity, or Single Cell.

Tubes

1

2

1.00 mL

0.50 mL

500000

750000

Max: 1,000,000 events

Max: 1,152,000 events

Assign a sort population by clicking a tube and selecting the population that you want.

T Regs

CD4+CD1...

Population Hierarchy

All Events

Scatter

SSC Singlets

FSC Singlets

CD4+

T Regs

CD4+CD127+CD25-

Select the initial buffer volume and the number of target events to be sorted into each tube(s).

Assign the sort population by clicking a tube and selecting the sort population from the Population Hierarchy.

Plates and slides

COLLECTION SETUP

Format: Plate

Number of wells: 96 well

Sort Mode: Single Cell

Enable Index Sort

(Optional) Select Enable Index Sort to perform an index sort on plates or slides.

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11

12

A

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C

D

E

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G

H

Unassign Selected

Select All

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Initial Buffer Volume: 0.00 mL

Number of Events: 10

Max: 79,200 events

Population Hierarchy

All Events

Scatter

SSC Singlets

FSC Singlets

P1

P2

Select the initial buffer volume (plates) or additive (slides) and the number of target events to be sorted into each well.

Select the sort population from the Population Hierarchy.

Assign the sort wells by clicking each well, dragging across a group of wells, clicking the letter or number for a row or column, or clicking Select All. You can also select non-contiguous wells by using Ctrl+click.

5

- 1 Click the **Sort** tab.

1 Design Experiment 2 View Data 3 Set Up Sort 4 Sort 5 View Reports

2 Insert the collection tubes into the appropriate tube holder.

3 Click **Start Sort**.

- 4 Monitor the sort by viewing the sort status and sort population plots.

The screenshot displays the FlowJo software interface, which is divided into several functional areas:

- Top Bar:** Indicates the acquisition is "RUNNING...". It includes a "Download Sample" button, a "Pause Sample" button, and a "Sort Status - Sorting..." section.
- Acquisition Parameters:**
 - Flow Rate: 100 (with a dropdown menu)
 - Total Events: 18,505
 - Processed Events: 99.48%
 - Elapsed Time: 00:32:15
 - Recording Criteria: 10,000 (with a dropdown menu)
 - Population: All Events (with a dropdown menu)
- Control Panel:**
 - Buttons for "Start Recording" (red circle), "Stop" (red square), "Pause Sort" (orange square), and "Aplation" (grey circle).
 - Buttons for "Off" (grey square) and "Light" (grey square).
 - A "Backflush" button.
- Sort Status - Sorting...:**
 - Buttons for "Stop Sort" (red square) and "Pause Sort" (orange square).
 - A "Extract" button.
 - Sort Mode: Purity
 - Remaining Time: 145 minutes
- Sort Population Plots:**
 - A grid of plots showing the sorting process. The top row shows "FSC Singlets" (a scatter plot of FSC-A vs. SSC-A) and "CD44" (a contour plot of CD44-APC vs. CD44-APC-A).
 - The bottom row shows "Scatter" (a scatter plot of SSC-A vs. SSC-A) and "SING Singlets" (a contour plot of SING-A vs. SING-A).
 - Each plot has a corresponding "All Events" plot to its right, showing the full population of events.
- Population Table:**

Population	Trips	CD4+CD127+CD25-
Target Count:	500000	750000
Sort Count:	846	846
Sort Rate:	85	85
Efficiency:	90.00	90.00
- Visual Representation:** Two vertical test tubes are shown at the bottom. The left tube contains green beads, and the right tube contains pink beads, representing the sorted populations.

[View reports](#)

- 1 Click the **View Reports** tab.

1 Design Experiment 2 View Data 3 Set Up Sort 4 Sort 5 View Reports

- 2 View the information and click **Export Report**.

Select Your Report
Sort_OO2
Sort_Report

Sort_OO2

CYTOMETER INFO

User Name: admin@admin
Experiment Name: TREC

Application Name: BD FACSCanto
Application Version: 1.11.10

Collection Method: None
Collection Name: FACS-Medley

VORT DETAILS

Sort Method: Purify
Sort Device: Tubes 50mL
Sort Events: 25,000
Processed Events: 1000%

Sort Status: Completed
Sort Size: 100 micron
Pressure: 25.73 PSI
Drop Frequency: 240 kHz

Statistics

Tuple	Stat	Target Count	Sort Count	Sort Rate	Efficiency	Time
1	P1	300	100	33%	9%	
2	P4	750	750	0	78%	0s

CYTOMETER SETTINGS

Fluorochrome: PMT Voltages
PSC: 328
SSC: 435
FITC: 483
Alma 647: 511
BV421: 503

Compensation: Spillover Values
Info (Detectors):

FITC: 100.00% 0.00 0.10
Alma 647: 0.00 100.00% 0.00
BV421: 0.00 0.00 100.00%

POPULATION HIERARCHY

All Events
SSC Singlets
FSC Singlets
CD45
CD4
CD34
CD34-CD137-CD45
P4
P5

Shut down system

You will be given an option to perform either Daily Shutdown or Long-Term Shutdown upon logging out or closing the application. You may also access these procedures through the Cytometer menu.

- 1 Click **Cytometer** from the navigation bar.
- 2 Click the **Daily Shutdown** or **Long-Term Shutdown** option.
- 3 Follow the prompts on the screen for each numbered step.

STARTUP / SHUTDOWN

System Startup

Prepares the cytometer for sorting by performing fluidics startup, cytometer setup (CS&T), and setting the drop delay.

CS&T Last Run: 03/14/2017 6:39 AM
Drop Delay Last Run: 03/14/2017 6:41 AM

Daily Shutdown

Cleans the sample path and fills the flow cell with BD Detergent Solution in preparation for shutdown.

Last Run: 03/13/2017 5:45 PM

Long-Term Shutdown

Removes sheath fluid from the lines, fills the lines with 70% ethanol, and drains the flow cell. Run this procedure when the cytometer will not be used for more than two days.

Last Run: N/A

Troubleshooting tips

See the Troubleshooting chapter in the *BD FACSMelody Cell Sorter User's Guide* for a complete list of topics and recommended solutions.

Observation	Possible causes	Recommended solutions
The stream has stopped or will not start. Stream error is displayed in the Status window.	Stream disruption due to air bubbles or unstable air pressure	Check that all fluid and air connectors are properly connected to the sheath and waste tanks. Make sure that the sheath-tank lid gasket is seated properly. Purge the sheath filter. Restart the stream by loading a sample.
	Sort nozzle is clogged	Sonicate the sort nozzle. Reinsert the nozzle and start the stream by loading a sample.
	Dirty strobe lens or upper camera window	Stop the stream and clean the strobe lens window and the upper camera window. Restart the stream by loading a sample.
No events seen in plots after clicking Load Sample or Start Sort.	Laser shutter is engaged	Make sure that the flow cell access door is closed.
Problems with Cytometer Setup function	Baseline or performance check failed	Prepare a new CS&T sample with the proper concentration as instructed in the product insert. Clean the flow cell. See <i>Cleaning the flow cell</i> in the <i>BD FACSMelody Cell Sorter User's Guide</i> . Close the sort block door and the flow cell door properly.
Problems with Drop Delay function	Sort block door is not closed	Close the sort block door properly. Clean the lower camera and Accudrop laser window.
	Event rate is too low or too high	Prepare a new Accudrop sample with the proper concentration as instructed in the technical data sheet.
	Debris on lower camera or Accudrop window	See <i>Cleaning the Accudrop laser window and lower camera window</i> in the <i>BD FACSMelody Cell Sorter User's Guide</i> .

Additional resources

Visit bdbiosciences.com and select Support > Training to view e-Learning videos. Additional information can also be found in the User's Guide accessible from the BD FACSCorus software Help menu.