BD FACSMelody™ System Quick Reference Guide

e-Learning: bdbiosciences.com/en-us/learn/training/self-paced-courses#e-learning-courses

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User's Guide: BD FACSChorus™ Software Help menu

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BD FACSMelody™ System Quick Reference Guide

This reference guide contains instructions for using the BD FACSMelody™ Cell Sorter with BD FACSChorus™ Software version 3.0. See the appropriate section in the user's guide for more detailed information.

Workflow Overview

The following shows a typical workflow when using the BD FACSMelody™ System.

Start up system ▸ Create experiment ▸ Set up and sort ▸ View sort reports ▸ Shut down system

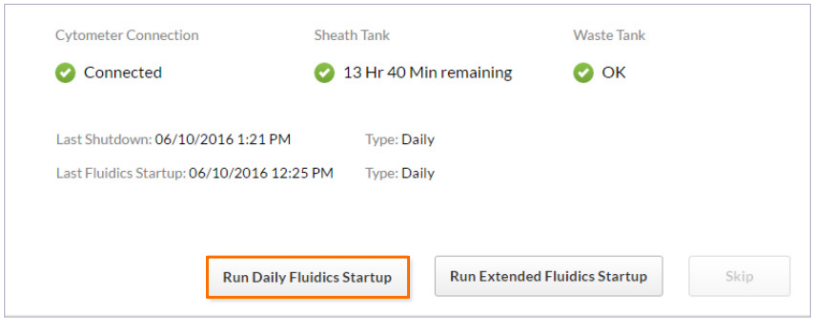
Start up system

Check fluids

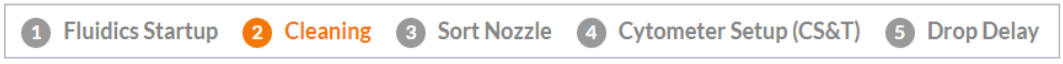
1. Fill the sheath tank to the weld line with sterile 1X phosphate-buffered saline (PBS).
   Note: Keep the sheath tank in the same location. If the location or elevation of the sheath tank is changed, it could affect the flow rate calibration.
2. Empty the waste tank and add approximately 1 L of undiluted bleach or a sufficient amount so that 10% of the total volume is bleach.

Fluidics startup

1. Verify that air is being supplied to the instrument via the compressor or house air source.
2. Press the power button on the front of the cell sorter unit.
3. Start BD FACSChorus™ Software by double-clicking the shortcut on the desktop and logging 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.
4. Once the system has connected, click **Run Daily Fluidics Startup**.



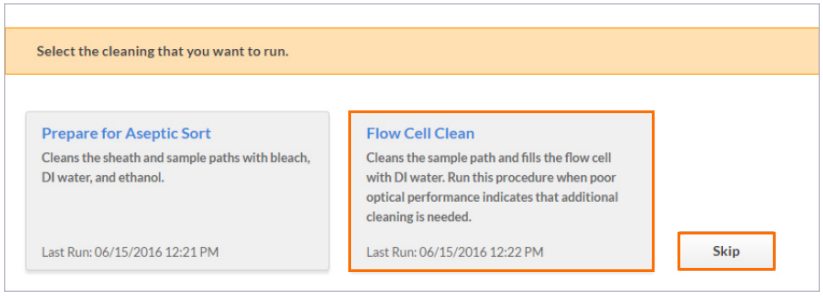
5. Follow the prompts on the screen for each numbered step. Be sure to insert the closed-loop nozzle with the O-ring facing up.
6. After fluidics startup is complete, click **Continue** to see the cleaning options.



Cleaning

Select the type of cleaning that you want to run. **Note:** This step can be skipped however we recommend that you perform the Flow Cell Clean Procedure after each startup.

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.



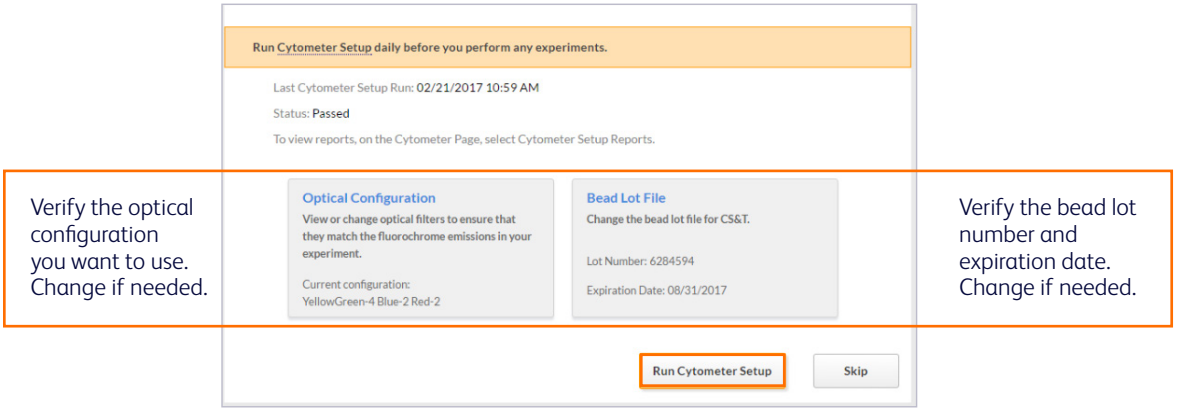
Sort nozzle

1. Insert the sort nozzle straight into the bottom of the flow cell cuvette with the orange O-ring and "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 (CS&T) and Drop Delay daily before performing any experiments.

1. Prepare a tube of BD® CS&T RUO Beads according to the package directions. **Note:** Do not dilute the beads with water.
2. Click **Run Cytometer Setup**.



3. Load the tube and follow the prompts.
4. After the CS&T process has completed successfully, prepare the BD FACS™ Accudrop RUO Beads according to the package directions and click **Continue** to run Drop Delay. **Note:** Do not dilute the beads with water.

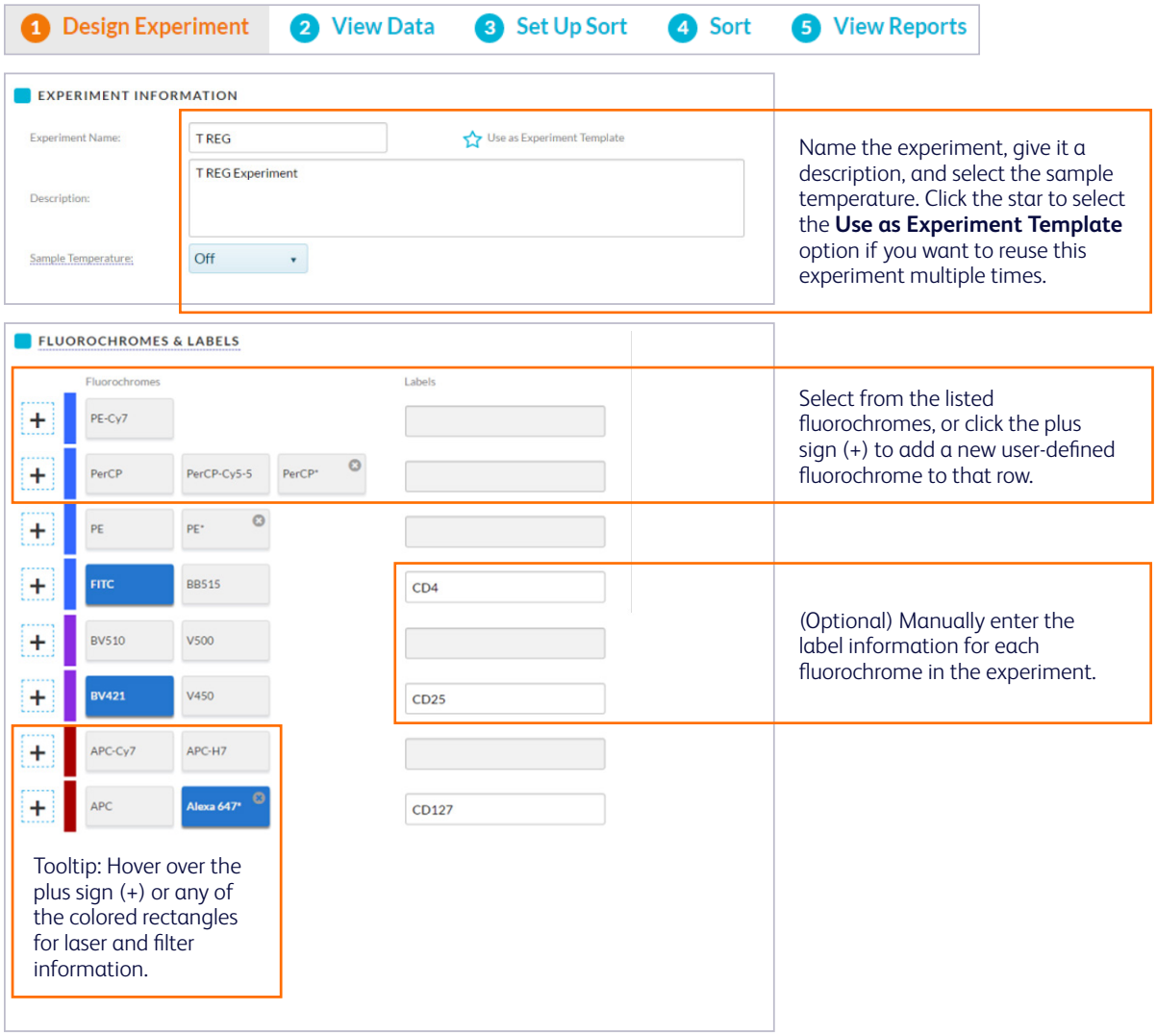
5. Load the tube and follow the prompts.

Create experiment

Experiments are used to define and refine the parameters for data acquisition and sorting.

Design experiment

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



View data

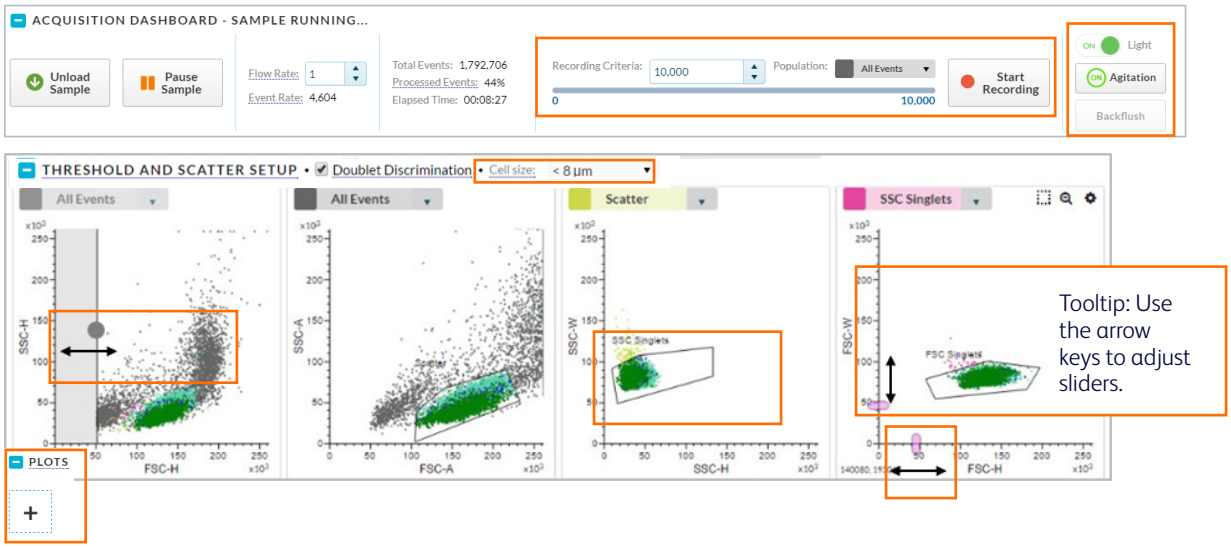
The selections on the View Data tab determine the layout of the experiment data. Optimize the threshold and scatter setup, then collect a pre-sort data file.

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

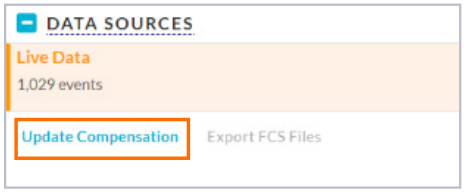
2. On the Acquisition dashboard, click **Load Sample** and adjust the flow rate as needed.
(Optional) Turn on the sample chamber light and agitation option.

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 stored spillover values will be used.

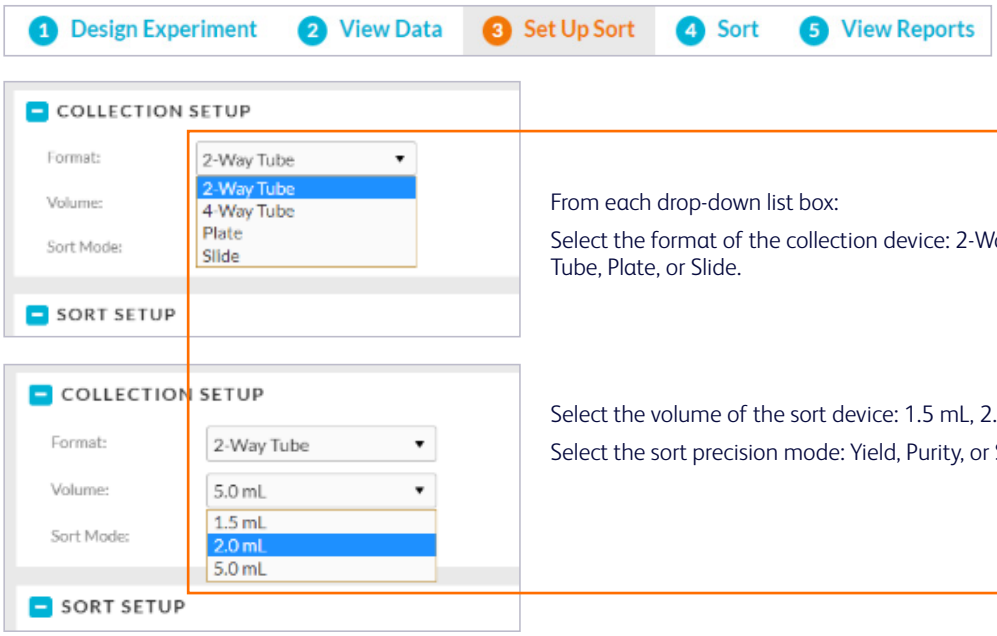


6. Select the Recording Criteria and click **Start Recording** on the acquisition dashboard to collect a pre-sort FCS data file.

Set up and sort

The selections on the Set Up Sort tab determine the collection device and the populations in the sample to be sorted.

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



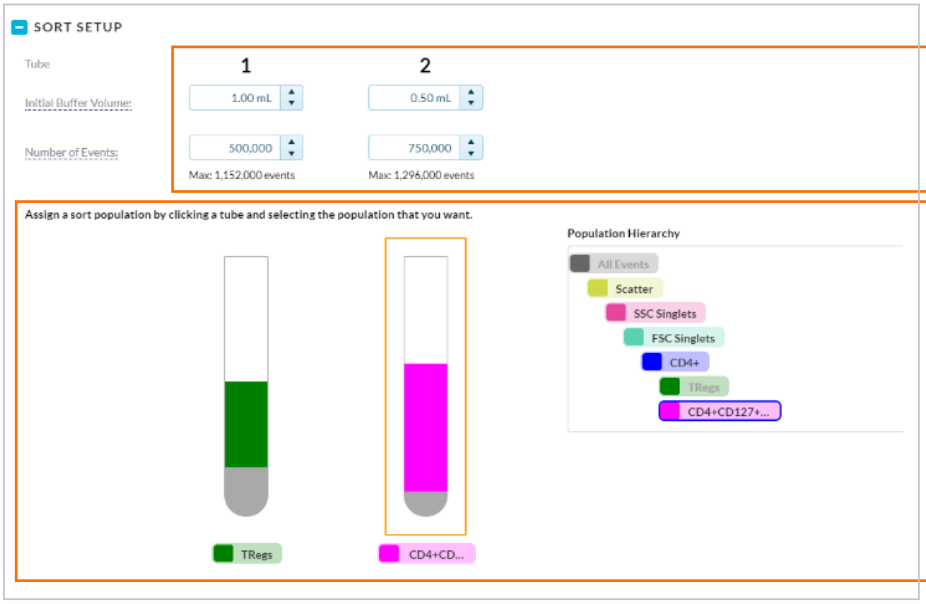
From each drop-down list box:

Select the format of the collection device: 2-Way Tube, 4-Way Tube, Plate, or Slide.

Select the volume of the sort device: 1.5 mL, 2.0 mL, or 5.0 mL.

Select the sort precision mode: Yield, Purity, or Single Cell.

Tubes: Two tubes view

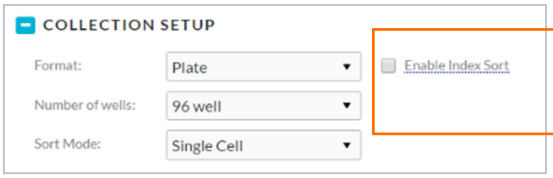


Select the initial buffer volume and the number of target events to be sorted into each tube.

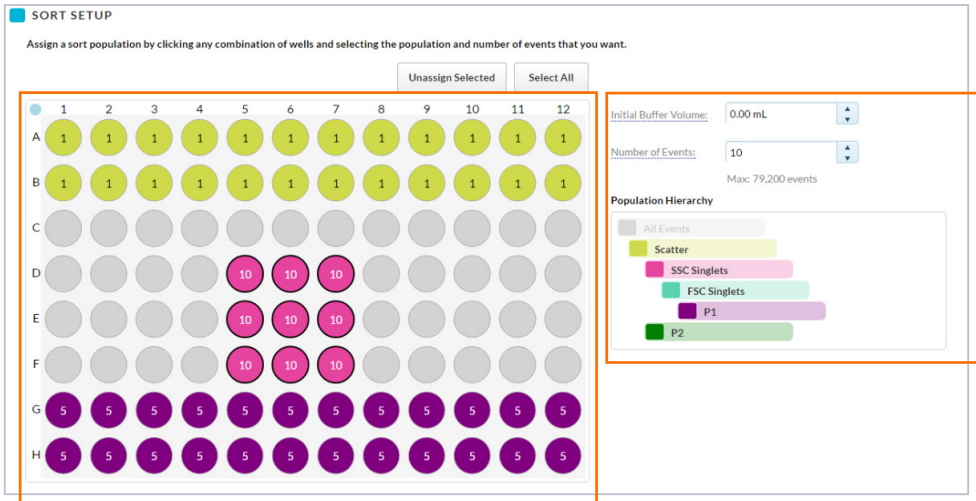
Assign the sort population by clicking a tube and selecting the sort population from the Population Hierarchy.

Tubes: Four tubes view

Plates and slides



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



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.

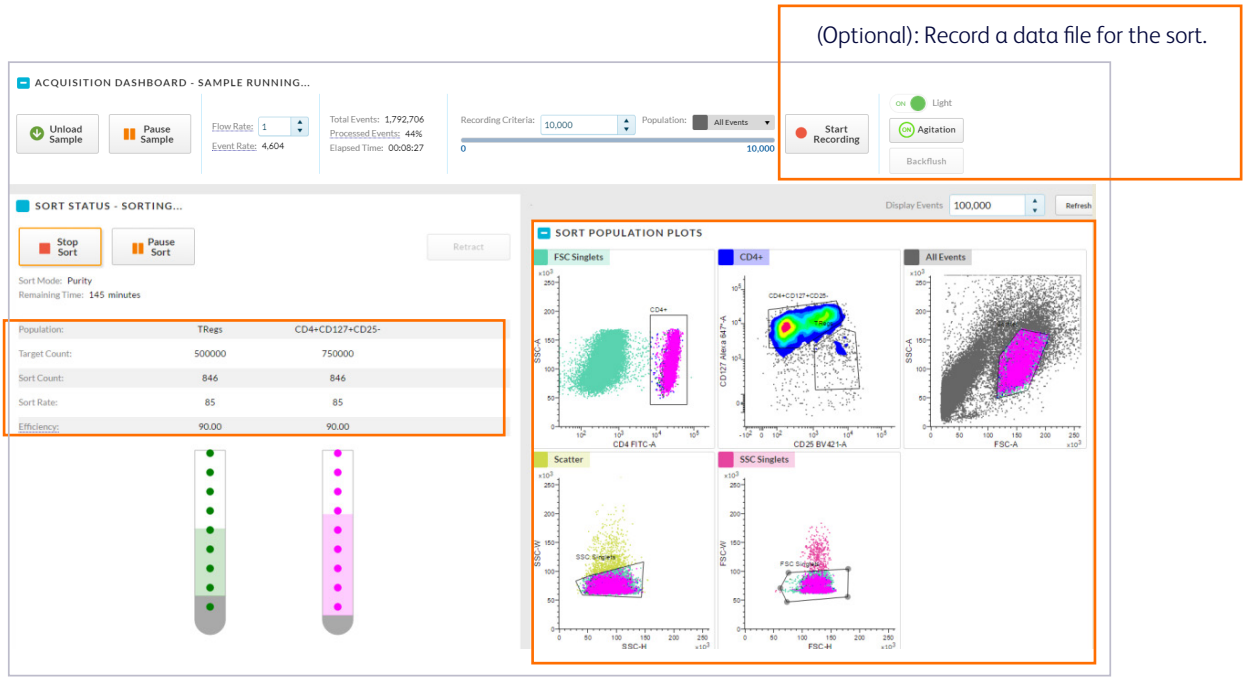
Sort

1. Click the **Sort** tab.

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.

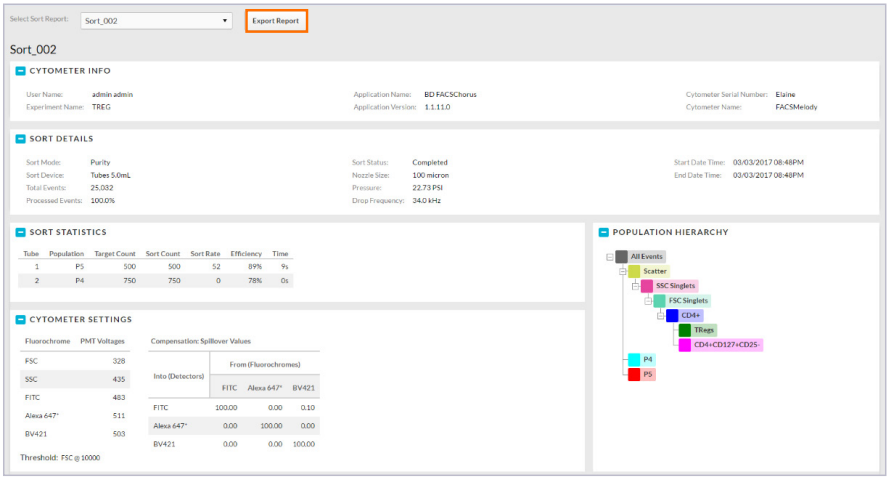


View sort reports

A sort report summarizing the results of the sort is displayed on the View Reports tab when sorting is complete.

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

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



Clean the Sample Line

This procedure cleans the sample line with a bleach solution. We recommend that you perform this at the end of your experiment and inbetween users.

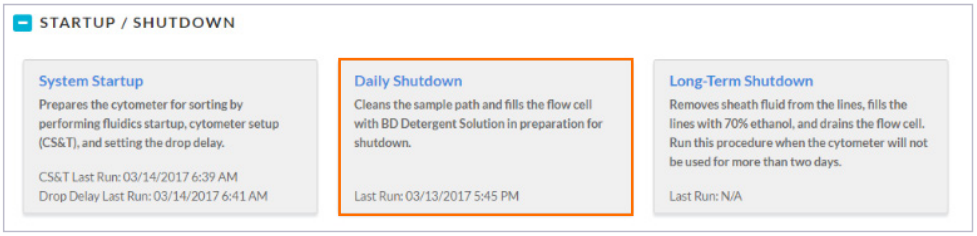
1. Load a tube containing 3 mL of a 10% bleach solution onto the sample loading port.
2. From the View Data tab, click **Load Sample**.
3. After approximately 5 minutes, click **Unload Sample**.
4. Load a tube containing 3 mL of DI water* onto the sample loading port.
5. Repeat steps 2 and 3.

* Note: It is very important to always run a tube of DI water after running bleach on the cell sorter.

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 can also access these procedures through the Cytometer menu. Note: Only use tanks that are provided with the BD FACSMelody™ System.

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



4. Power off the cytometer unit.

Troubleshooting tips

BD FACSChorus™ Software provides some troubleshooting instructions when errors are encountered. The tips in this section are focused on errors or troubleshooting that the software is not able to address and designed to help you troubleshoot your experiments. If additional assistance is required, contact your local BD Biosciences technical support representative. See the appropriate section in the user's guide for complete instructions on how to perform the recommended solutions.

Startup troubleshooting

Observation	Possible causes	Recommended solutions
Closed loop nozzle is not detected	Salt buildup on the closed-loop nozzle	Clean the closed loop nozzle.
	Salt buildup in the nozzle location between the flow cell and the locking lever	Clean the area to remove the salt buildup.
Error starting stream after inserting sort nozzle or loading sample	Sheath tank low or empty, or waste tank full or almost full	Fill the sheath tank to the maximum level or empty the waste tank.
	Sort nozzle inserted improperly	Remove the nozzle and ensure that the O-ring is in place. Re-insert the nozzle. Make sure the nozzle is dry.
	Dirty strobe lens or upper camera window	Clean the lens and the window as described in Cleaning the strobe lens window and upper camera window.
	Clogged or damaged sort nozzle	Turn off the stream, remove the nozzle, and examine the nozzle tip under a microscope. If debris is visible, clean the nozzle. If the nozzle seems damaged, replace it. Restart the stream.
	Debris in flow cell	Scrub the flow cell.
Error starting stream after inserting sort nozzle or loading sample	Air in sheath line or filter	Stop and restart the stream. Purge the sheath filter. Run daily fluidics startup.
	Dry sheath filter	Purge the sheath filter.
	Air pressure is too low, too high, or variable	Verify that the external air supply or compressor is on and the pressure is between 80 and 95 psi. Verify that the sheath tank lid is sealed properly.
	Residual ethanol in fluidic lines	Run extended fluidics startup.
	Sheath filter orientation is incorrect.	Change the orientation of the filter.

Observation	Possible causes	Recommended solutions
Stream not in center of waste aspirator drawer	Sort nozzle inserted improperly	Remove the nozzle and ensure that the O-ring is in place. Re-insert the nozzle.
	Clogged or damaged sort nozzle	Turn off the stream, remove the nozzle, and examine the nozzle tip under a microscope. If debris is visible, clean the nozzle. If the nozzle seems damaged, replace it. Restart the stream.
	New sort nozzle was inserted.	If you are using a new nozzle, the sort block might need to be repositioned to align with the stream.
	Air bubbles in flow cell	Stop and restart the stream to remove bubbles.
	– Ethanol or other cleaning solution in flow cell – Dirty flow cell	Scrub the flow cell.
Prepare for Aseptic Sort fails	Fluid or air lines are detached	Verify that the fluid or air line connections are attached. Push firmly on each line to ensure that it is connected.
Problems with Cytometer Setup function	Baseline or performance check failed, or stopped before completing	Prepare a new CS&T sample with the proper concentration as instructed in the product insert. Close the sort block door and the flow access door properly. Clean the flow cell. Confirm that fluid is flowing through the sample line by checking that the fluid levels in the sample tube have decreased. If the fluid levels in the sample tube have not decreased, massage the sample line. If sample flow seems to be blocked, then backflush the sample line several times to clear the block. Clean the flow cell again and check that the fluid levels in the sample tube have decreased. If sample flow continues to be blocked, change the sample line filter. Perform clean flow cell again and check that the fluid levels in the sample tube have decreased.
Problems with Cytometer Setup function	– Beads not on scale – Low event rate or zero event rate	Prepare a new CS&T sample with the proper concentration as instructed in the product insert. **Note:** Do not dilute BD® CS&T RUO Beads with water. Close the sort block door and the flow access door properly. Turn off the stream and remove, sonicate, and reinsert the nozzle. Clean the flow cell. Confirm that fluid is flowing through the sample line by checking that the fluid levels in the sample tube have decreased. If the fluid levels in the sample tube have not decreased, massage the sample line. If sample flow seems to be blocked, then backflush the sample line several times to clear the block. Clean the flow cell again and check that the fluid levels in the sample tube have decreased. If sample flow continues to be blocked, change the sample line filter. Perform clean flow cell again and check that the fluid levels in the sample tube have decreased.
BD FACS™ Accudrop laser scan fails to locate stream after the nozzle is changed.	Steam is unable to focus or software fails to detect that the nozzle was changed.	Check the stream. If necessary, adjust sort block so that the stream is in the center of the waste aspirator. See Aligning the waste aspirator drawer to the stream in the user's guide. Restart the workstation to trigger software detection of the new nozzle.
Lower (stream) camera does not show laser/stream	Steam is unable to focus or software fails to detect that the nozzle was changed.	Check the stream. If necessary, adjust sort block so that the stream is in the center of the waste aspirator. See Aligning the waste aspirator drawer to the stream in the user's guide. Restart the workstation to trigger software detection of the new nozzle.

Acquisition troubleshooting

Observation	Possible causes	Recommended solutions
Problems with Drop Delay function	Sort block door is not closed	Close the sort block door properly.
	Flow cell access door is open	Close the flow cell access door properly.
	Event rate is too low or too high	Prepare a new Accudrop sample with the proper concentration as instructed in the technical data sheet. **Note:** Do not dilute BD FACS™ Accudrop RUO with water. Clean the flow cell. Confirm that fluid is flowing through the sample line by checking that the fluid levels in the sample tube have decreased. If the fluid levels in the sample tube have not decreased, massage the sample line to clear a possible sample line blockage. If sample flow seems to be blocked, backflush the sample line several times to clear the block. Clean the flow cell again and check that the fluid levels in the sample tube have decreased. If sample flow continues to be blocked, change the sample line filter. Clean the flow cell again and check fluid levels in the sample tube. If sample flow continues to be blocked, replace the sample line. Clean the flow cell again and check fluid levels in the sample tube.
	Debris on lower camera or Accudrop window	Clean the lower camera and Accudrop laser window.
No events in plots or events don't update in plots after clicking Load Sample	Selected data source is a recorded file	Select the Live Data data source.
	Laser shutter is engaged	Close the flow cell access door properly.
	No sample in the tube	Add sample to the tube or install a new sample tube.
	Sample line or sample line filter is clogged	Clean the flow cell. Confirm by checking that fluid levels in the sample tube have decreased. If the fluid levels in the sample tube have not decreased, massage the sample line. If sample flow seems to be blocked, backflush the sample line several times to clear the block. Clean the flow cell again and check fluid levels in the sample tube. If sample flow continues to be blocked, change the sample line filter. Clean the flow cell again and check fluid levels in the sample tube. If sample flow continues to be blocked, replace the sample line. Clean the flow cell again and check fluid levels in the sample tube.
	Sample is not mixed properly	Resuspend the sample. Turn on or increase the sample agitation rate.
	Threshold is not set to correct parameter	Set the threshold to the correct parameter for your application.
	Threshold setting is too low or too high	Adjust the threshold setting.

Observation	Possible causes	Recommended solutions
Unexpected events in plots or fewer events in gated populations than expected	Incorrect logic in population hierarchy	Verify the gating strategy.
	Threshold not set to correct parameter	Set the threshold to the correct parameter for your application.
	Threshold setting is too low or too high	Adjust the threshold setting.
	Events left out of a gate	When drawing a gate, make sure that events on the axes are included.
	Cell size is set incorrectly	Ensure that the setting for the cell size is appropriate for your sample.
	Sample preparation is inadequate	Ensure that your tubes are clean prior to sample addition, re-stain a new sample, and follow standard protocols for preparing your specific sample type.
Erratic event rate	Sample is not adequately mixed or is aggregated	Filter the sample. Resuspend the sample. Turn on or increase the sample agitation rate.
	Sheath tank is low	Fill the sheath tank.
	Sample preparation is inadequate	Ensure that your tubes are clean prior to sample addition, re-stain a new sample, and follow standard protocols for preparing your specific sample type.
	Sample chamber O-ring is worn	Contact your BD Biosciences field service engineer.
Unexpectedly high event rate	Sample is not adequately mixed or is aggregated	Filter the sample. Resuspend the sample. Turn on or increase the sample agitation rate.
	Threshold setting is too low	Adjust the threshold setting.
	Sample is too concentrated	Dilute the sample.
	Flow rate is too high	Decrease the flow rate.
	Bubbles in flow cell	Turn off the stream, wait a few seconds, and then load the sample again. Scrub the flow cell.
Unexpectedly low event rate	Sample is not adequately mixed or is aggregated	Filter the sample. Resuspend the sample. Turn on or increase the sample agitation rate.
	Sample is too dilute	Concentrate the sample.
	Threshold setting is too high	Adjust the threshold setting.
	Sample line assembly or sample line filter installed incorrectly	Verify the sample line assembly or sample line filter installation.
	Sample line is clogged or kinked	If visible kinks are found in the sample line, replace the sample line assembly. If visible kinks are not found in the sample line, clean the flow cell. Confirm that fluid is flowing through the sample line by checking that the fluid levels in the sample tube have decreased. If the fluid levels in the sample tube have not decreased, massage the sample line. If sample flow seems to be blocked, backflush the sample line several times to clear the block. Clean the flow cell again and check that the fluid levels in the sample tube have decreased. If sample flow continues to be blocked, change the sample line filter. Clean the flow cell again and check that the fluid levels in the sample tube have decreased.

Observation	Possible causes	Recommended solutions
Distorted populations or high CVs	Instrument settings adjusted incorrectly	Optimize the threshold setting, voltage settings, and run user-defined compensation to optimize compensation settings.
	Flow rate is too high	Decrease the flow rate.
	Bubbles in flow cell	Turn off the stream, wait a few seconds, and then load the sample again.
	Debris in flow cell or nozzle	Scrub the flow cell with BD® Detergent Solution. Remove the nozzle, and examine the nozzle tip under a microscope. If debris is visible, clean the nozzle.
	Sample is not adequately mixed or is aggregated	Filter the sample. Resuspend the sample. Turn on or increase the sample agitation rate.
	Sample preparation is inadequate	Ensure that your tubes are clean prior to sample addition, re-stain a new sample, and follow standard protocols for preparing your specific sample type.
	Sheath filter is more than 3 months old	Replace the sheath filter.
Excessive amount of debris in plots	Threshold setting is too low	Adjust the threshold setting.
	Dead cells or debris in sample	Examine the sample under a microscope to determine the source of the debris. Adjust sample preparation if needed.
	Sample preparation is inadequate	Ensure that your tubes are clean prior to sample addition, re-stain a new sample, and follow standard protocols for preparing your specific sample type.
	Sheath filter needs to be replaced	Replace the sheath filter.
Processed events are <90%	Threshold setting is too low	Adjust the threshold setting.
	Event rate is too high	Decrease the flow rate.
	Sample is not adequately mixed or is aggregated	Filter the sample. Resuspend the sample. Turn on or increase the sample agitation rate.
Stream turns off unexpectedly	Nozzle clog detected or debris in nozzle	Remove the nozzle, and examine the nozzle tip under a microscope. If debris is visible, clean the nozzle.
	Debris in flow cell	Follow the scrub the flow cell procedure with 1.5% BD® Detergent Solution.
	Sheath tank empty or waste tank full	Empty the waste tank or fill the sheath tank.
Unable to start sort	BD FACSChorus™ software cannot locate the side streams	Clean the lower camera window. Close the sort block door properly. When using four-way sort, wait for a few minutes to allow the Accudrop to find the four streams. If the streams are still not found, clean the nozzle. Also, clean the deflection plates.
	Salt bridge	Clean the deflection plates and the area around and behind the plates.

Observation	Possible causes	Recommended solutions
Arcing between deflection plates	Sort nozzle inserted improperly	Remove the nozzle and ensure that the O-ring is in place. Re-insert the nozzle.
	Clogged or damaged sort nozzle	Turn off the stream, remove the nozzle, and examine the nozzle tip under a microscope. If debris is visible, clean the nozzle. If the nozzle seems damaged, replace it. Restart the stream.
	Dirty deflection plates	Clean the deflection plates.
	Particles too big for sort nozzle	Verify that the particle size is appropriate for the 100-µm nozzle. In general, the nozzle orifice should be at least 5 times the average particle size in the sort sample. See Shapiro H. *Practical Flow Cytometry*. Fourth Edition. New York, NY: John Wiley and Sons; 2003:263.
Low sort efficiency	Event rate is too high for drop frequency	Decrease the flow rate.
	Incorrect sort mode	Verify that the sort mode is appropriate for your sorting requirements.
	Gating conflict	Verify the gating hierarchy.
Erratic sort rate	Flow rate is too high	Decrease the flow rate.
Unexpected sort results	Incorrect drop delay	Run drop delay.
	Incorrect sort mode	Verify that the sort mode is appropriate for your sorting requirements.
	Incorrect logic in population hierarchy	Verify the gating hierarchy. Do not assign conflicting gates (for example, parent population in Tube 1, child population in Tube 2).
Plate sorting failure	Splash shield not installed	Install the splash shield.
	Sort collection chamber door is open	Close the sort collection chamber door.
	Automated stage does not move	Close the access doors, then restart the instrument and workstation.
Unable to sort into targeted well in plate	Debris on deflection plates	Clean deflection plates.
	Waste aspirator drawer not aligned to stream	Align the waste aspirator drawer.
	Automated stage improperly aligned	Align the stage. If the problem cannot be resolved by aligning the automated stage, contact your BD service representative for assistance.

Electronics troubleshooting

Observation	Possible causes	Recommended solutions
Cell sorter will not connect to workstation	Cell sorter power is off	Turn on the cell sorter main power.
	Ethernet cable between workstation and cell sorter is disconnected	Unplug and then plug in the cable and make sure it is secure.
	IP address or other connectivity information changed.	Call BD Biosciences for assistance.

Maintenance tasks

Category	Task	When to perform
Shutdown	Clean the sample line	At the end of each experiment and between users.
	Daily shutdown	At the end of any given day the system is being used. You can also perform this cleaning separately whenever additional cleaning of the sample path and flow cell is needed.
	Long-term shutdown	Perform every 6 months and when the system will be off for more than 2 days.
Update compensation standards	Update the normalized spillover values	Run this procedure with BD® FC Beads every 60 days.
Nozzle and flow cell	Clean the sort and/or closed-loop nozzle	When you see indications of clogging or salt buildup.
	Clean the flow cell	Perform separately whenever additional cleaning is needed, and in cases where debris builds up in the flow cell as indicated by high CVs in the CS&T report. See procedure for cleaning the flow cell in the user's guide.
	Align the waste aspirator drawer to the stream	If you install a sort nozzle that is new or different from the one that came with the instrument.
Fluidics	Replace the waste filter cap	Monthly.
	Change the fluid filter	Every 3 months or as needed.
	Purge the sheath filter	Perform as a task after installing a new sheath filter and whenever you observe problems with the stream.
	Replace the sample line	Every 4-6 months or when decreased event rates indicate that the sample line might be clogged.
	Backflush the sample line	When you observe sample carryover, or after you run samples with adherent cells or dye.
	Replace the sample line filter	When decreased event rates indicate that the sample line might be clogged.
	Align the automated stage	After replacing a damaged sort nozzle, when using a sheath fluid other than PBS, or whenever it is especially important that each drop falls in the exact center of the well.
Optics	Clean the deflection plates	When you have trouble viewing the side stream or after a clog.
	Clean the Accudrop laser window and the lower camera window	When the software is unable to set drop delay, or when the software is unable to verify the side streams when sorting is started.
	Cleaning the strobe lens window and upper camera window	When smudges appear in the Stream View window, after a clog, or after sheath fluid has leaked or sprayed.

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