

# BD FACSMelody™ System Quick Reference Guide



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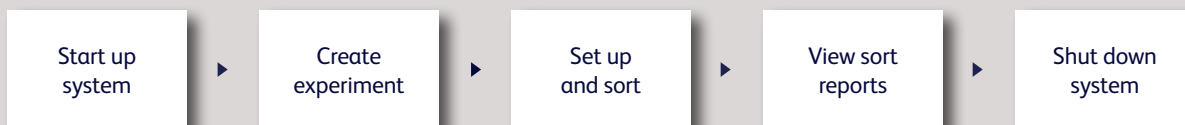
**User's Guide:** BD FACSCorus™ Software Help menu

# BD FACSMelody™ System Quick Reference Guide

This reference guide contains instructions for using the BD FACSMelody™ Cell Sorter with BD FACSCorus™ 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.



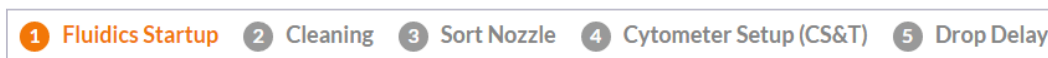
## Start up system

### 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 FACSCorus™ 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**.



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

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

**Run Daily Fluidics Startup**    Run Extended Fluidics Startup    Skip

- 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.

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 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 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 according to the package directions. **Note:** Do not dilute the beads with water.
- 2 Click **Run Cytometer Setup**.

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.

**Optical Configuration**  
View or change optical filters to ensure that they match the fluorochrome emissions in your experiment.  
Current configuration:  
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 optical configuration you want to use. Change if needed.

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

Run Cytometer Setup Skip

- 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.

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

- 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.

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

### EXPERIMENT INFORMATION

Experiment Name:  ★ Use as Experiment Template

Description:

Sample Temperature:

Name the experiment, give it a description, and select the sample temperature. Click the star to select the **Use as Experiment Template** option if you want to reuse this experiment multiple times.

### FLUOROCHROMES & LABELS

| Fluorochromes   | Labels                             |
|---|------------------------------------|
| <input type="checkbox"/> PE-Cy7   | <input type="text"/>               |
| <input type="checkbox"/> PerCP <input type="checkbox"/> PerCP-Cy5-5 <input type="checkbox"/> PerCP* | <input type="text"/>               |
| <input type="checkbox"/> PE <input type="checkbox"/> PE*  | <input type="text"/>               |
| <input checked="" type="checkbox"/> FITC <input type="checkbox"/> BB515                             | <input type="text" value="CD4"/>   |
| <input type="checkbox"/> BV510 <input type="checkbox"/> V500  | <input type="text"/>               |
| <input type="checkbox"/> BV421 <input type="checkbox"/> V450  | <input type="text" value="CD25"/>  |
| <input type="checkbox"/> APC-Cy7 <input type="checkbox"/> APC-H7                                    | <input type="text"/>               |
| <input type="checkbox"/> APC <input checked="" type="checkbox"/> Alexa 647*                         | <input type="text" value="CD127"/> |

Select from the listed fluorochromes, or click the plus sign (+) to add a new user-defined fluorochrome to that row.

(Optional) Manually enter the label information for each fluorochrome in the experiment.

Tooltip: Hover over the plus sign (+) or any of the colored rectangles for laser and filter information.

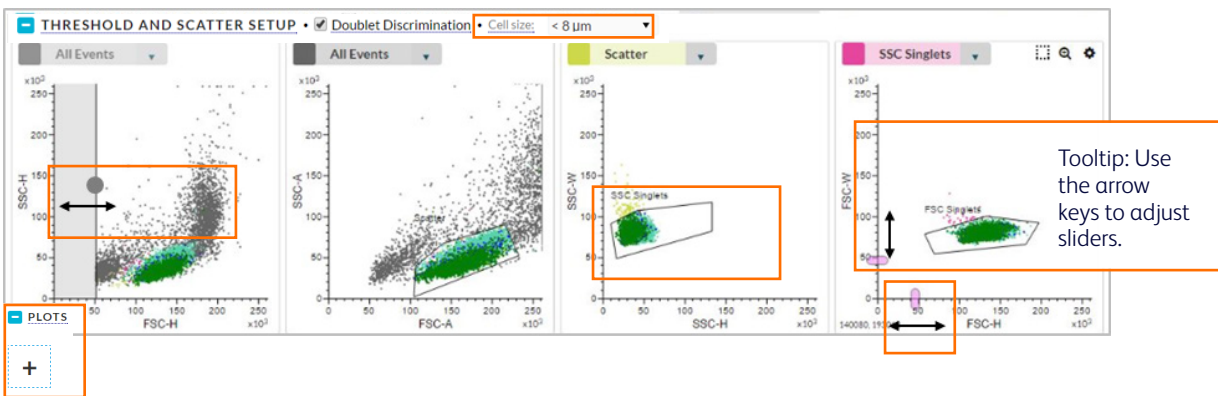
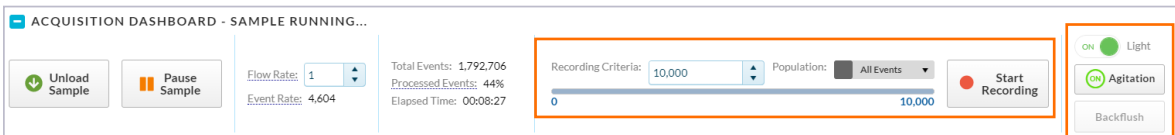
## View data

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.

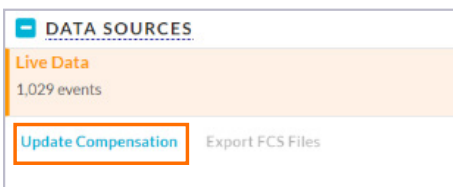
- 1 Click the **View Data** tab.



- 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.

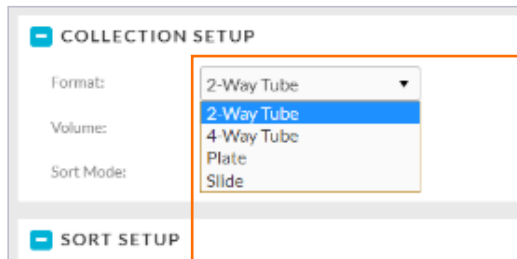


- 6 Select the Recording Criteria and click **Start Recording** on the acquisition dashboard to collect a pre-sort FCS data file.

## Set up and sort

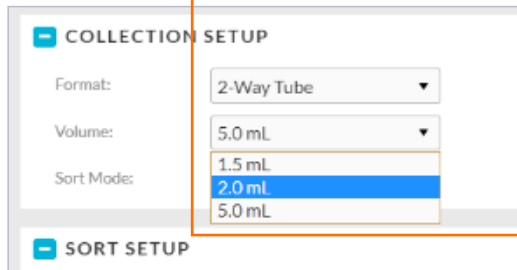
The selections on the Set Up Sort tab determine the collection device and the populations in the sample to be sorted.

- 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

**Sort Setup**

Tube

| 1  | 2                                  | 3                                  | 4                                  |
|--|------------------------------------|------------------------------------|------------------------------------|
| Initial Buffer Volume: 0.00 mL                       | 0.00 mL                            | 0.00 mL                            | 0.00 mL                            |
| Number of Events: 1,296,000<br>Max: 1,296,000 events | 1,296,000<br>Max: 1,296,000 events | 1,296,000<br>Max: 1,296,000 events | 1,296,000<br>Max: 1,296,000 events |

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

Population Hierarchy

- All Events
- Scatter
- SSC Singlets
- FSC Singlets
- Combined
- P1
- FITC
- Unstained
- APC
- PerCP

## Plates and slides

**Collection Setup**

Format:

Number of wells:

Sort Mode:

[Enable Index Sort](#)

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

**Sort Setup**

Assign a sort population by clicking any combination of wells and selecting the population and number of events that you want.

Unassign Selected | Select All

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.

## 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.

(Optional): Record a data file for the sort.

ACQUISITION DASHBOARD - SAMPLE RUNNING...

Unload Sample | Pause Sample | Start Recording

Flow Rate: 1 | Total Events: 1,792,706 | Recording Criteria: 10,000 | Population: All Events  
 Event Rate: 4,604 | Processed Events: 44% | Elapsed Time: 00:08:27

Sort Status - SORTING...  
 Stop Sort | Pause Sort | Retract

Sort Mode: Purity  
 Remaining Time: 145 minutes

| Population:   | TRegs  | CD4+CD127+CD25- |
|---------------|--------|-----------------|
| Target Count: | 500000 | 750000          |
| Sort Count:   | 846    | 846             |
| Sort Rate:    | 85     | 85              |
| Efficiency:   | 90.00  | 90.00           |

SORT POPULATION PLOTS

- FSC Singlets
- CD4+
- All Events
- Scatter
- SSC Singlets

## 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**.

Select Sort Report: Sort\_002 | Export Report

Sort\_002

**CYTOMETER INFO**  
 User Name: admin admin | Application Name: BD FACSCorus | Cytometer Serial Number: Ewino  
 Experiment Name: TREG | Application Version: 1.11.11.0 | Cytometer Name: FACSMelody

**SORT DETAILS**  
 Sort Mode: Purity | Sort Status: Completed | Start Date Time: 03/03/2017 08:48PM  
 Sort Device: Tubes 5.0mL | Nozzle Size: 100 micron | End Date Time: 03/03/2017 08:48PM  
 Total Events: 25,002 | Pressure: 22.73 PSI  
 Processed Events: 100.0% | Drop Frequency: 342.4Hz

**SORT STATISTICS**

| Tube | Population | Target Count | Sort Count | Sort Rate | Efficiency | Time |
|------|------------|--------------|------------|-----------|------------|------|
| 1    | P5         | 500          | 500        | 52        | 89%        | %    |
| 2    | P4         | 750          | 750        | 0         | 78%        | 0s   |

**CYTOMETER SETTINGS**

| Fluorochrome | PMT Voltages | Compensation: Spillover Values |
|--------------|--------------|--------------------------------|
| FSC          | 328          | From (Fluorochromes)           |
| SSC          | 435          | Into (Detectors)               |
| FITC         | 483          | FITC                           |
| Allexa 647   | 511          | Allexa 647                     |
| BV421        | 503          | BV421                          |

Threshold: FSC @ 10000

**POPULATION HIERARCHY**

- All Events
  - Scatter
    - FSC Singlets
      - SSC Singlets
        - CD4+
        - Tregs
        - CD4+CD127+CD25-



## 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.

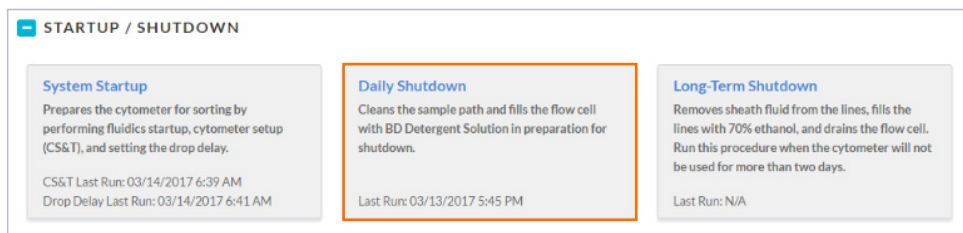
- 1 Load a tube containing 3 mL of a 10% bleach solution onto the sample loading port.
- 2 From the View Data tab, click **Load Sample**.
- 3 After approximately 5 minutes, click **Unload Sample**.
- 4 Load a tube containing 3 mL of DI water\* onto the sample loading port.
- 5 Repeat steps 2 and 3.

\* 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.



The screenshot shows a menu titled "STARTUP / SHUTDOWN" with three options:

- 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.

- 4 Power off the cytometer unit.

## Troubleshooting tips

BD FACSCorus™ 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.<br>If debris is visible, clean the nozzle.<br>If the nozzle seems damaged, replace it.<br>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.<br>Purge the sheath filter.<br>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.<br>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.<br>If debris is visible, clean the nozzle.<br>If the nozzle seems damaged, replace it.<br>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<br>– 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.<br>Close the sort block door and the flow access door properly.<br>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.<br>If the fluid levels in the sample tube have not decreased, massage the sample line.<br>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.<br>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<br>– Low event rate or zero event rate                        | Prepare a new CS&T sample with the proper concentration as instructed in the product insert. <b>Note:</b> Do not dilute BD® CS&T RUO Beads with water.<br>Close the sort block door and the flow access door properly.<br>Turn off the stream and remove, sonicate, and reinsert the nozzle.<br>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.<br>If the fluid levels in the sample tube have not decreased, massage the sample line.<br>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.<br>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. | Stream is unable to focus or software fails to detect that the nozzle was changed. | Check the stream.<br>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.<br>Restart the workstation to trigger software detection of the new nozzle.  |
| Lower (stream) camera does not show laser/stream                                 | Stream is unable to focus or software fails to detect that the nozzle was changed. | Check the stream.<br>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.<br>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            | <p>Prepare a new Accudrop sample with the proper concentration as instructed in the technical data sheet. <b>Note:</b> Do not dilute BD FACS™ Accudrop RUO with water.</p> <p>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.</p> <p>If the fluid levels in the sample tube have not decreased, massage the sample line to clear a possible sample line blockage.</p> <p>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.</p> <p>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.</p> <p>If sample flow continues to be blocked, replace the sample line. Clean the flow cell again and check fluid levels in the sample tube.</p> |
|   | 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 | <p>Clean the flow cell. Confirm by checking that fluid levels in the sample tube have decreased.</p> <p>If the fluid levels in the sample tube have not decreased, massage the sample line.</p> <p>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.</p> <p>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.</p> <p>If sample flow continues to be blocked, replace the sample line. Clean the flow cell again and check fluid levels in the sample tube.</p>   |
|   | Sample is not mixed properly                 | <p>Resuspend the sample.</p> <p>Turn on or increase the sample agitation rate.</p>  |
|   | 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.<br>Resuspend the sample.<br>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.<br>Resuspend the sample.<br>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.<br>Scrub the flow cell.   |
| Unexpectedly low event rate   | Sample is not adequately mixed or is aggregated                  | Filter the sample.<br>Resuspend the sample.<br>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.<br>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.<br>If the fluid levels in the sample tube have not decreased, massage the sample line.<br>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.<br>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.<br>Remove the nozzle, and examine the nozzle tip under a microscope.<br>If debris is visible, clean the nozzle.   |
|                                     | Sample is not adequately mixed or is aggregated       | Filter the sample.<br>Resuspend the sample.<br>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.<br>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.<br>Resuspend the sample.<br>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.<br>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 FACSCorus™ software cannot locate the side streams | Clean the lower camera window.<br>Close the sort block door properly.<br>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.<br>If debris is visible, clean the nozzle.<br>If the nozzle seems damaged, replace it.<br>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- $\mu$ m nozzle. In general, the nozzle orifice should be at least 5 times the average particle size in the sort sample. See Shapiro H. <i>Practical Flow Cytometry</i> . 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.<br>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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