

Job Aid

BD FACSDiscover™ S8 Cell Sorter: Performing a system startup with an extended fluidics startup

This job aid contains instructions for how to perform an extended fluidics startup for the BD FACSDiscover™ S8 System. Selecting the appropriate fluidics startup depends on which shutdown procedure was previously completed. For additional information, see the *BD FACSDiscover™ S8 Cell Sorter with BD CellView™ Image Technology and BD SpectralFX™ Technology User's Guide*.

- If a long-term shutdown was performed, you must perform an extended fluidics startup.
- If a daily shutdown was performed and a daily fluidics startup was not completed within 24 hours, the system recommends an extended fluidics startup.



Materials needed

The following items are needed to complete a system startup:

- BD FACSDiscover™ Setup Beads
- Bleach (5% sodium hypochlorite)
- Sterile Deionized (DI) water
- BD FACSFlow™ Sheath Fluid or sterile 1X PBS
- BD FACS™ Accudrop Beads
- BD CellView™ Calibration Beads

Before you begin

Ensure that the in-house external air supply or optional air compressor is turned on, the air is supplied to the system between 5.5–5.9 Bar (80 and 95 psi), the sheath tank is full, and the waste tank is empty.

Starting up the system

Turning on the cytometer and workstation

NOTE If you are working with a biological safety cabinet (BSC) or an aerosol management system (AMO), ensure it is turned on for 3 minutes before switching on the cell sorter.

1. Press the power button on the right side of the upper cell sorter unit.

NOTE Ensure the instrument is switched on for at least 30 minutes before use.

2. Press the power button on the PC workstation, and log into Microsoft® Windows®.
3. Open BD FACSCorus™ Software by double-clicking the shortcut on the desktop and logging in with your user information.

The software connects with the cell sorter within 5 minutes. The Connected status in the Status Indicator panel will initially be yellow when the software connects with the instrument. After the connection is established successfully, the Connected status turns green.



Filling the sheath tank

1. Disconnect the clear air line by holding the top of the air line and pulling up on the lower air connector port to release the connection.
2. Vent the air pressure from the sheath tank by pulling the metal ring on the pressure-relief valve.
3. Unscrew the sheath tank cover knob and remove the cover.
4. Fill the sheath tank to the weld line with BD FACSTFlow™ Sheath Fluid (non-sterile) or sterile/non-sterile 1X phosphate-buffered saline (PBS).

NOTE Do not overfill the sheath tank because it can cause incorrect sample flow rates.

5. Replace the cover and tighten the knob.

NOTE To avoid damage to the O-ring, do not over-tighten the knob to seal the lid.

6. Reconnect the air line by pushing the air line into the connector until you hear a click.



Emptying the waste tank

1. Disconnect the waste sensor and liquid waste line from the fluid storage cart's respective labeled ports and remove the tank.



2. Unscrew the disposable waste cap (large cap) and the attached waste trap from the tank. Place the assembly on the bench with the label side up.

NOTE Do not wet the cap on top of the trap.

3. Remove the waste tank from the cart and dispose of the waste according to your standard laboratory procedures for biohazardous waste.

NOTE Do not add bleach to the waste tank if you are performing an extended startup since ethanol in the system from a long-term shutdown will be flushed into the waste tank.

4. Replace the disposable waste cap (large cap) and the attached waste trap, then tighten them by hand until they are closed.

NOTE Do not overtighten the trap or attached filter cap. Tighten each component only until it is hand-tight. Do not use sealants or adhesives.

5. Reconnect the waste liquid line and waste sensor to the respective ports on the fluid storage cart.



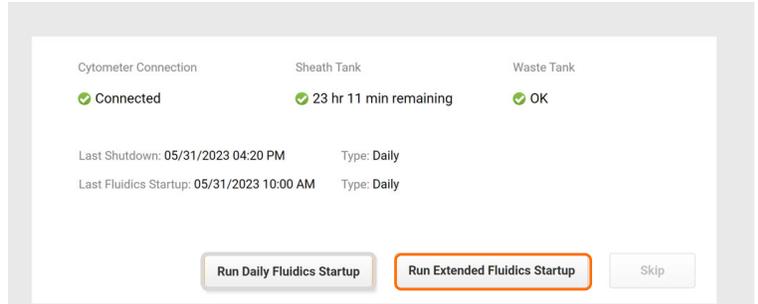
Running an Extended Fluidics Startup

Initiating a fluidics startup

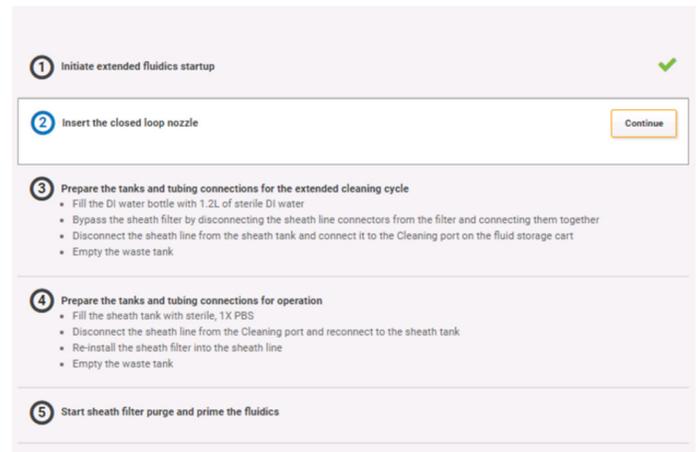
- 1 Fluidics Startup 2 Cleaning 3 Sort Nozzle 4 Setup and QC 5 Image Calibration 6 Drop Delay

1. Click **Run Extended Fluidics Startup**.

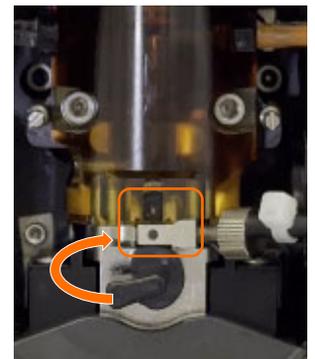
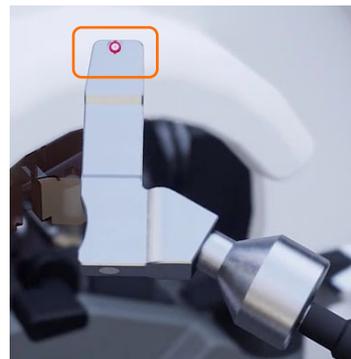
NOTE You must run an Extended Fluidics Startup after a long-term shutdown.



2. Follow the prompts on the screen through each numbered step.



- a. At prompt 2, to prepare for cleaning, insert the closed-loop nozzle into the bottom of the flow cell cuvette with the O-ring facing up.
- b. Close the sort chamber door, then click **Continue**.



Initiating a fluidics startup, continued

b. To prepare the bottles, tanks and tubing connections for the extended cleaning cycle by doing the following:

- i. Fill the white DI water bottle on the fluid cart with 1.2 L of sterile DI water.
- ii. Bypass the sheath filter by disconnecting the blue sheath line connectors from the top and bottom of the filter and connecting them together.

iii. Disconnect the blue sheath line from the sheath tank and connect it to the cleaning port of the fluid storage cart.

iv. Empty the waste tank.

CAUTION Do not add bleach to the waste tank if you at this step since ethanol in the system from a long-term shutdown will be flushed into the waste tank.

v. Click **Continue**.

5. To prepare the tanks and tubing connections for normal operation.

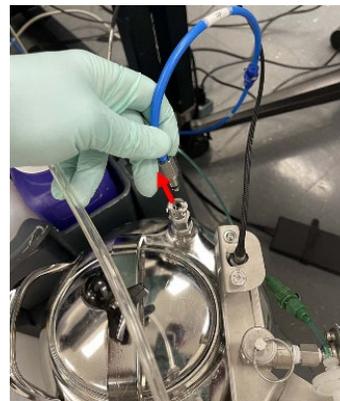
- a. Fill the sheath tank with sterile, 1X PBS or BD FACSTow™ Sheath Fluid.
- b. Disconnect the sheath line from the cleaning port and reconnect it to the sheath tank.
- c. Re-install the sheath filter into the sheath line.
- d. Empty the waste tank and add approximately 1 L of undiluted bleach to the waste tank or enough so that 10% of the total volume is bleach.
- e. Click **Continue**.

6. At prompt 5, click **Continue** to start a sheath filter purge and prime the fluidics.

7. Click **Close** and then **Continue** to proceed to the next step in the System Startup workflow: Cleaning.

3 Prepare the tanks and tubing connections for the extended cleaning cycle

- Fill the DI water bottle with 1.2L of sterile DI water
- Bypass the sheath filter by disconnecting the sheath line connectors from the filter and connecting them together
- Disconnect the sheath line from the sheath tank and connect it to the Cleaning port on the fluid storage cart
- Empty the waste tank



4 Prepare the tanks and tubing connections for operation

- Fill the sheath tank with sterile, 1X PBS
- Disconnect the sheath line from the Cleaning port and reconnect to the sheath tank
- Re-install the sheath filter into the sheath line
- Empty the waste tank

Cleaning the flow cell

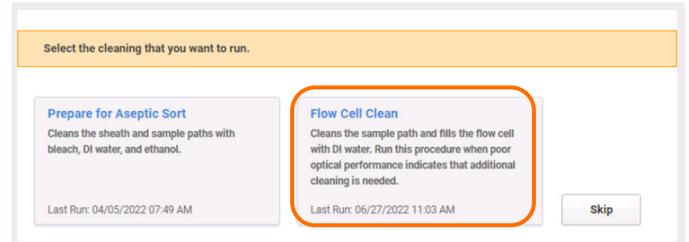
- 1 Fluidics Startup 2 **Cleaning** 3 Sort Nozzle 4 Setup and QC 5 Image Calibration 6 Drop Delay

We recommend running this Flow Cell Clean during the startup procedure.

1. Click **Flow Cell Clean** and follow the prompts on the screen through each numbered step.

NOTE For additional cleaning, use 1.5% BD® Detergent Solution instead of DI water.

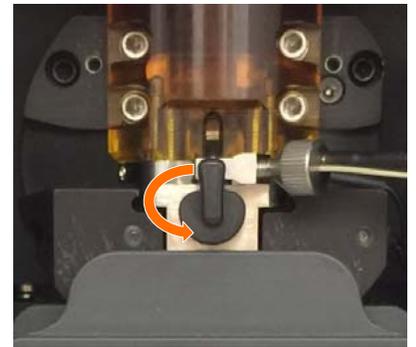
2. After cleaning is complete, click **Close**, then **Continue** to proceed to the next step in the Startup workflow: Sort Nozzle.



Inserting the Sort Nozzle

- 1 Fluidics Startup 2 Cleaning 3 **Sort Nozzle** 4 Setup and QC 5 Image Calibration 6 Drop Delay

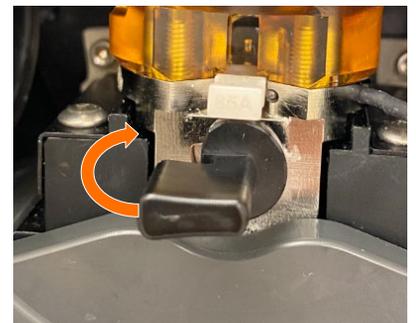
1. Remove the closed-loop nozzle by turning the nozzle-locking lever counterclockwise to the 9 o'clock position, pull the nozzle straight out and place it in the holder.



2. Insert a sort nozzle straight into the bottom of the flow cell cuvette with the orange O-ring facing up.



3. Turn the nozzle-locking lever clockwise to the 12 o'clock position, click **Continue** to start the stream, and proceed to the next step in the startup workflow, Setup and QC.



Running a Setup and QC

- 1 Fluidics Startup
- 2 Cleaning
- 3 Sort Nozzle
- 4 Setup and QC
- 5 Image Calibration
- 6 Drop Delay

Baseline: A baseline setup must be run when you select a new bead lot to set the basic performance criteria for the cell sorter. It should also be run if specific procedures on the cell sorter might have altered its performance. A baseline run takes more than 10 minutes to complete.

Daily Performance: A daily performance run should be performed each day you run the system to ensure that your cell sorter performs consistently over time. A daily performance run takes about 7–10 minutes to complete.

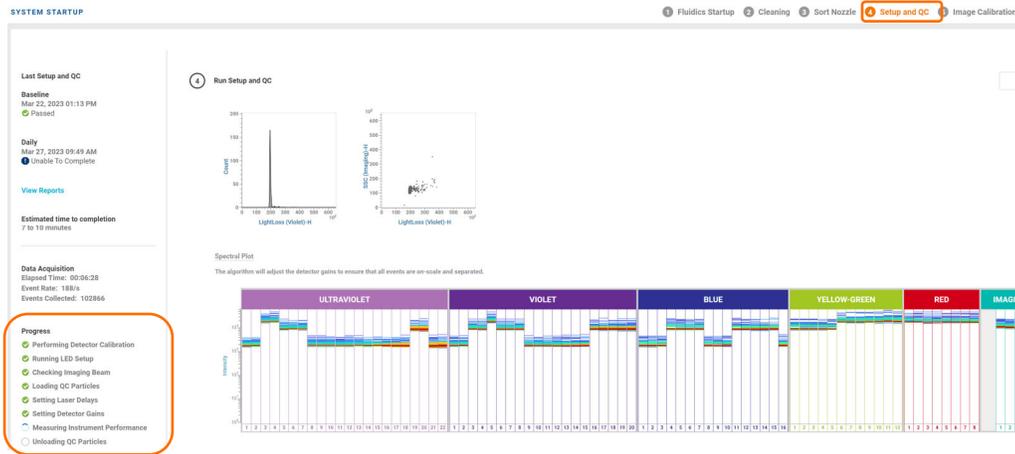
1. Prepare a tube of BD FACSDiscover™ Setup Beads according to the package insert.
2. Verify the bead lot file by expanding the drop-down on the right and selecting a bead lot (indicated by the bead lot number).

NOTE To add a new bead lot file, see Downloading and importing BD FACSDiscover™ Setup Beads bead lot files in the user's guide.

3. Load a tube of BD FACSDiscover™ Setup Beads.
4. Close the sample input and sort collection chamber doors.
5. Select **Daily** and click **Run**.

The screenshot displays the BD SYSTEM STARTUP interface. At the top, a progress bar shows six steps: 1 Fluidics Startup, 2 Cleaning, 3 Sort Nozzle, 4 Setup and QC (highlighted with an orange box), 5 Image Calibration, and 6 Drop Delay. On the left, a navigation menu includes Experiments, Cytometer, Help, and Joe (user profile), with a Log Out button at the bottom. The main content area is titled 'Last Setup and QC' and shows a 'Baseline' run from Mar 22, 2023, which passed. Below that, a 'Daily' run from Apr 11, 2023, is shown as 'Completed With Warnings'. A 'View Reports' link is present. At the bottom left, it states 'Estimated time to completion: 7 to 10 minutes'. The right side of the interface contains four numbered steps for the current 'Setup and QC' process: 1. 'Select a bead lot' with a dropdown menu showing '600915SU2' (highlighted with an orange box); 2. 'Load a tube with BD FACSDiscover™ Setup Beads'; 3. 'Select type of Setup and QC' with radio buttons for 'Daily' (selected and highlighted with an orange box) and 'Baseline'; 4. 'Run Setup and QC' with a 'Run' button (highlighted with an orange box).

The steps progress sequentially, and a spinning circle indicates the current step. When Setting Laser Delays begins, events start appearing on the plots. The system unloads the tube after the steps are completed or if there is an error during the setup process.



- Click **Continue** to proceed to Startup workflow: Image Calibration.

Running the Image Calibration

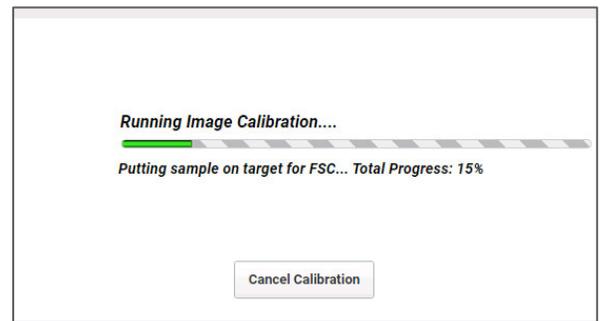
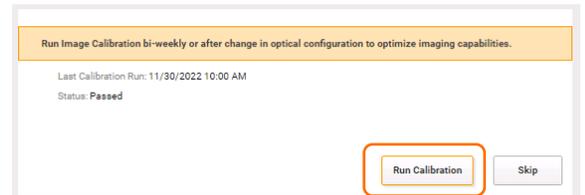
- Fluidics Startup
- Cleaning
- Sort Nozzle
- Setup and QC
- Image Calibration**
- Drop Delay

BD CellView™ Calibration Beads are used to optimize the imaging capability of the BD FACSDiscover™ S8 cell sorter. Run the image calibration every two weeks or for certain imaging troubleshooting steps.

- Prepare the BD CellView™ Calibration Beads according to the package insert.
- Click **Run Calibration**.
- Load a tube of BD CellView™ Calibration Beads and click **Continue**.

The system displays a progress bar and identifies the various stages of the process.

- When the completed successfully dialog appears, click **Continue** to proceed to Startup workflow: Drop Delay.

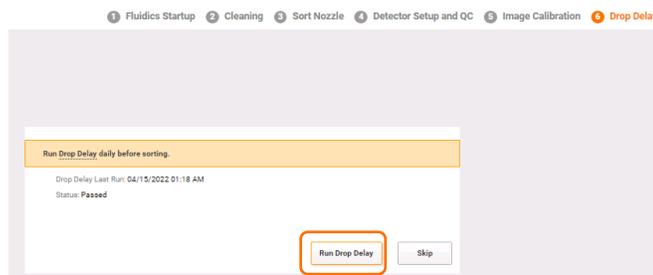


Setting the Drop Delay

- 1 Fluidics Startup
- 2 Cleaning
- 3 Sort Nozzle
- 4 Setup and QC
- 5 Image Calibration
- 6 Drop Delay

BD FACS™ Accudrop Beads automatically set an accurate drop delay value. Run drop delay daily or when you change the nozzle before a sort. This step can be skipped if the nozzle's drop delay has already been run today.

1. Prepare the BD FACS™ Accudrop Beads according to the package insert.
2. Click **Run Drop Delay**.



3. Load a tube with BD FACS™ Accudrop Beads and click **Continue**.

The system displays a progress bar and identifies the various stages of the process as they progress.

When the drop delay is completed, a completion dialog is displayed.

4. Click **Continue** to go to the Experiments page.

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