Job Aid

BD FACSCelesta™ Flow Cytometer: Performing system startup and shutdown with FFSS and HTS options

This job aid provides instructions to help you get started with using your cytometer. It contains instructions for performing system startup and shutdown with the BD FACSFlow™ Supply System (FFSS) and the BD® High Throughput Sampler (HTS) option. For additional information, see the appropriate cytometer user's guide.

Materials needed

The following materials are needed to complete the system startup and shutdown.

- BD FACS™ Sheath Solution with Surfactant Cat. No. 336524 (20L)
- BD FACSClean™ Solution Cat. No. 340345 (5L)
- BD® Detergent Solution Concentrate Cat. No. 660585
- Bleach
- DI water
- 12x75 mm round-bottom tubes

System startup

Turning on the system

1. Turn on the computer and log in to the operating system.

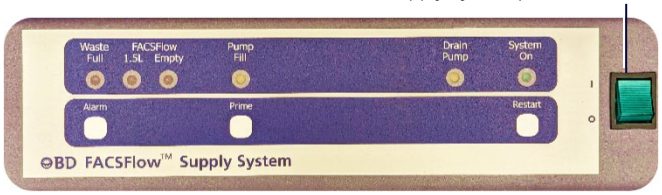
2. Verify the BD FACSFlow™ Supply System is on.

   NOTE If the cytometer is run with a BD FACSFlow™ Supply System that is powered off or improperly connected:

   - The sheath plenum can run dry, compromising data collection.
   - The waste container can overflow.

   To avoid these hazards, check the sheath supply and turn on the BD FACSFlow™ Supply System before turning on the cytometer.

BD FACSFlow™ Supply System power button



3. Press the power button on the right side of the instrument to turn on the cytometer main power.

4. Open BD® Coherent Connection Software to start the lasers.

   Most lasers will start automatically when the control software starts. For the UV laser, ensure the correct power is set and then start it manually.

Turning on the system, continued

5. Open BD FACSDiva™ Software.

6. Enter your log in information and then click **OK.**

7. Ensure that the software is connected (Cytometer Connected) to the cytometer by checking the bottom of the Cytometer window.

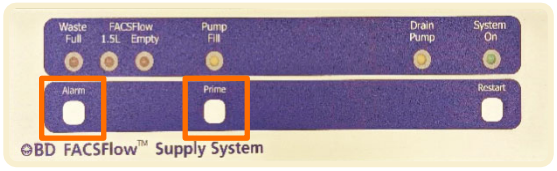
   If necessary, select **Cytometer > Connect.**

Checking the sheath fluid levels

1. Verify that the system status on the control panel is set to HTS MODE.

   - If needed, press and hold the Mode button for at least 3 seconds to change the mode.
   - If needed, silence the alarm by pressing Alarm on the BD FACSFlow™ Supply System panel.

   NOTE When the system is in HTS mode, both visual and audible alarms on the cytometer are deactivated. The fluid level alarms on the BD FACSFlow™ Supply System will be used instead.



2. Check for fluid in the sheath plenum. If the plenum is dry, fill it with approximately 1.5 L of sheath fluid by pressing and holding Prime button on the BD FACSFlow™ Supply System until enough sheath is added to the plenum.

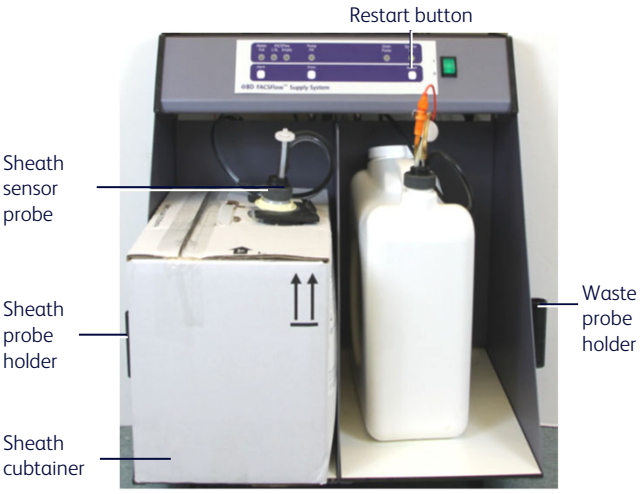
3. If the sheath cubitainer needs replacement, loosen the sheath sensor probe cap assembly and carefully remove the sensor probe from the sheath cubitainer, keeping it at a 45-degree angle.

4. Place the sensor probe into the sheath probe holder on the side of the BD FACSFlow™ Supply System unit.

5. Remove the cap and load the new sheath cubitainer into the left side of the unit.

6. Insert the sensor probe at a 45-degree angle into the sheath cubitainer and tighten the cap assembly.

7. Press Restart on the BD FACSFlow™ Supply System panel to reset the alarm.



Emptying the waste

1. Remove the cap assembly and level probe. Place the probe into the waste probe holder.

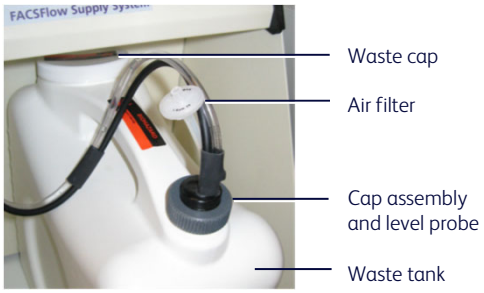
2. Remove the waste cap and place it label side up.

   NOTE Do not get the waste cap wet.

3. Empty the waste tank according to your standard laboratory procedures for biohazardous waste.

4. Add 1 L of bleach to the waste tank (a sufficient quantity of bleach to maintain at least 10% concentration of bleach when full). Reinstall the waste cap.

5. Retighten the cap assembly on the waste tank.



Checking for air bubbles

1. Check the sheath filter for trapped air bubbles.

2. If bubbles are visible, gently tap the filter body to dislodge the bubbles and force them to the top.

3. Direct the vent line into a beaker and press the small button at the end of the vent fitting against the side of the beaker until a steady stream of fluid empties from the filter.

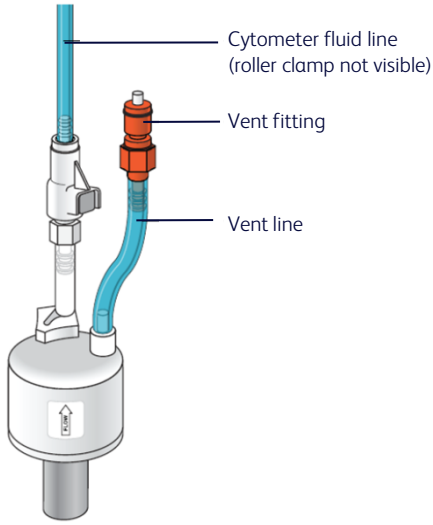
4. Tilt the filter and verify that no trapped air remains in the filter.

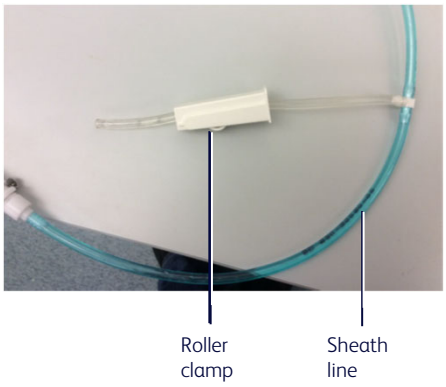
5. Repeat steps 3 and 4 until no air is observed in the filter.

6. Check the sheath line for air bubbles.

7. Open the roller clamp at the sheath filter to bleed off any air in the line. Use a beaker to collect the sheath fluid that is expelled.

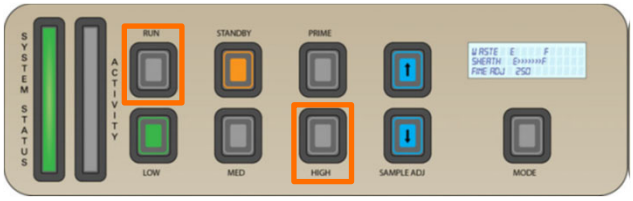
8. Close the roller clamp.





Rinsing the system

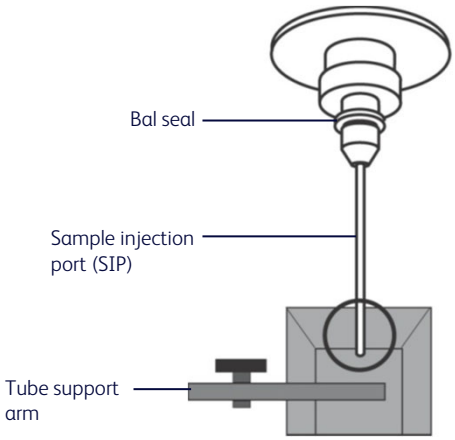
1. Set the sample fine adjust to 250, if needed.



2. Press RUN and HIGH on the cytometer fluidics control panel.

3. Install a tube containing 3 mL of 1.5% BD® Detergent Solution on the SIP and center the tube support arm under the tube.

   NOTE The RUN button turns green when the tube has pressurized. If the button remains amber, check the seal on the sheath tank or plenum, and check for a cracked tube or a worn Bal seal.



4. Allow the solution to run on high for 10 minutes.

5. Install a tube containing 3 mL of DI water on the SIP. Allow the solution to run on high for 10 minutes.

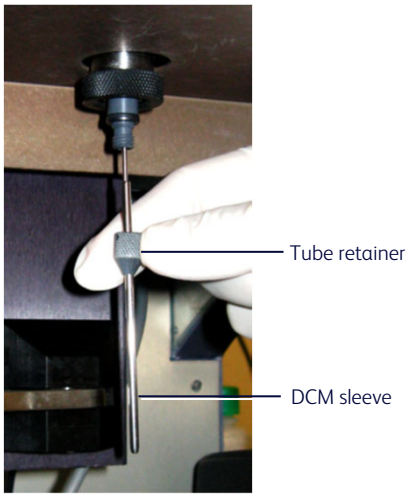
6. Press the STANDBY button on the fluidics control panel and place a tube containing no more than 1 mL of DI water on the SIP.

Preparing for plate-based acquisition

If your cytometer is already set up for plate-based acquisition, skip this section. Prior to performing the steps in this section, ensure you have the sample injection tube (SIT) protector available.

1. Remove the tube of DI water from the cytometer and place the tube support arm to the side.

2. Remove the droplet containment module (DCM) sleeve by unscrewing the tube retainer and carefully removing the sleeve.



3. Attach the SIT protector.

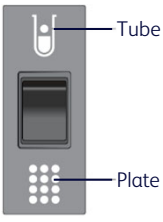
   a. Slide the SIT protector up over the SIT until it reaches a hard stop.

   b. Push up on the tube retainer until you can screw it onto the SIP.

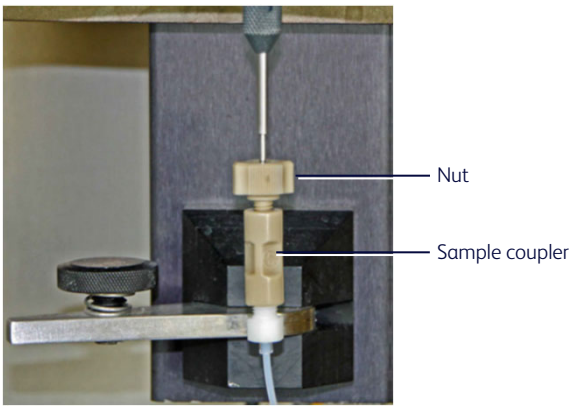
   c. Hand tighten the tube retainer.

4. Switch the acquisition mode switch to plate mode.

   The acquisition mode switch is on the right side of the cytometer.



5. Attach the HTS sample coupler to the cytometer SIT.
    a. Loosen the top nut from the sampler coupler.
    b. Slide the top nut onto the SIT followed by the lower portion of the sample coupler. Ensure that the sample coupler tubing is not kinked or twisted.
    c. Hold the coupler with one hand while you tighten the top nut with the other hand.
    d. Gently tug the sample coupler to make sure it is on tight.



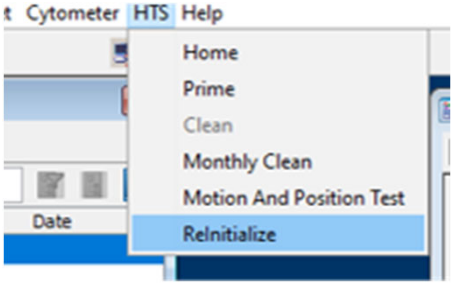
Initializing the HTS

To prevent bubble formation in the flow cell when acquiring samples with the HTS, use only BD FACS™ Sheath Solution with Surfactant.

1. Verify that the HTS power switch is in the ON position.

    If the HTS power switch is in the ON position, it will automatically turn on when the cytometer is turned on.

2. Install the HTS cover.



3. Press RUN on the cytometer fluidics control panel.

4. In BD FACSDiva™ Software, select **HTS > ReInitialize.**

    The HTS probe performs a homing sequence during initialization.

    NOTE If the HTS does not initialize, verify that the cytometer is in run mode and that the HTS cover is aligned.

5. Check the sheath filter and sheath line for bubbles. Purge air, if needed.

6. Prime the HTS fluidics.
    a. Verify the cytometer is in run mode.
    b. Select **HTS > Prime.**
    c. Repeat step b two more times.
    d. Verify that there are no visible bubbles in the HTS syringes or the green tubing. If you see air bubbles, repeat step b until no air bubbles can be seen.

7. Ensure that the sample coupler is not leaking.

8. Press STANDBY on the cytometer fluidics control panel.

System shutdown

Perform the cleaning procedure at the end of every day when using the HTS.

- If you will not use the HTS within the next 24 hours, complete this entire procedure.
- If you will use the HTS again within the next 24 hours, then skip the *Priming the HTS with water* section.

During the daily cleaning procedure, the cytometer uses cleaning solution and DI water from predefined wells and performs a sequence of mixing, aspirating, and rinsing. Software prompts guide you through the cleaning sequence. Allow 15 minutes to complete this procedure.

Wells	Amount	Solution or Sample	Function
A1 – A4	250 µL	BD FACSClean™ Solution	Clean
B1 – B4	250 µL	DI water	Rinse

NOTE If necessary, a 10% bleach solution can be used instead of BD FACSClean™ Solution.

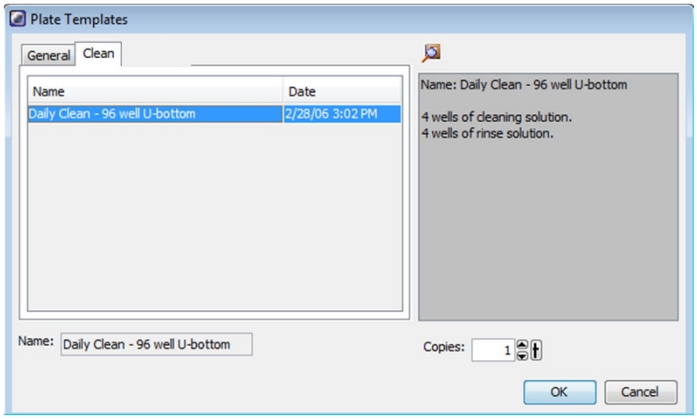
⚠ To ensure that the 10% bleach solution retains its full germicidal effect, prepare a fresh solution daily.

Performing daily clean with sheath fluid

1. Select **HTS > Clean.**

   The Plate Templates dialog appears.

2. Select the Daily Clean - 96 well U-bottom template, if not already selected.



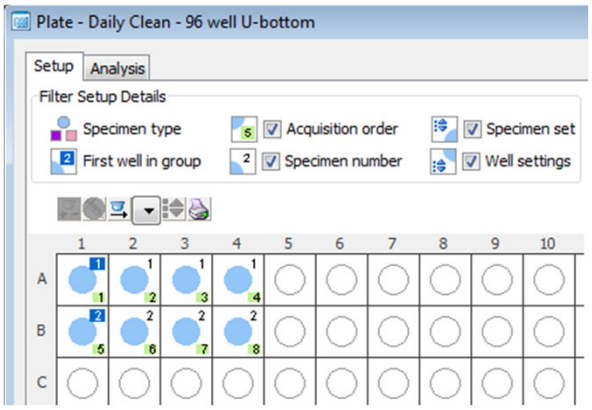
3. Click **OK.**

   The plate layout changes to show the Daily Clean Setup view.

4. Place the plate with cleaning solution and DI water on the HTS.

5. Click **OK** in the confirm dialog to begin the daily cleaning protocol.

   The HTS goes through a homing sequence, and cleaning begins. The cleaning procedure can take up to 15 minutes.

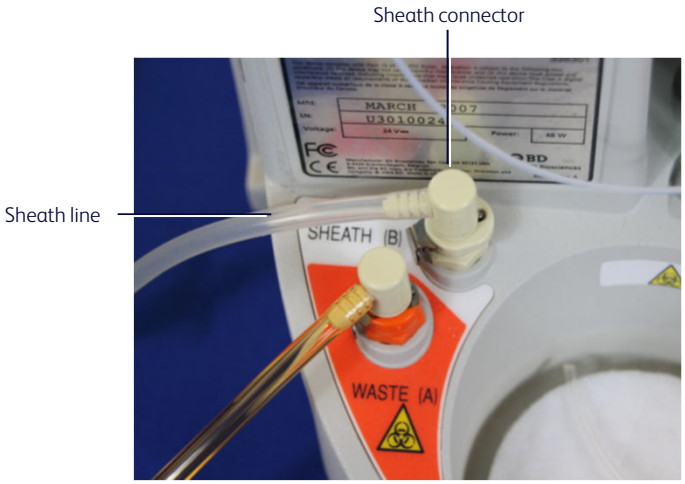


6. Click **OK** when the completion message appears.

Priming the HTS with water

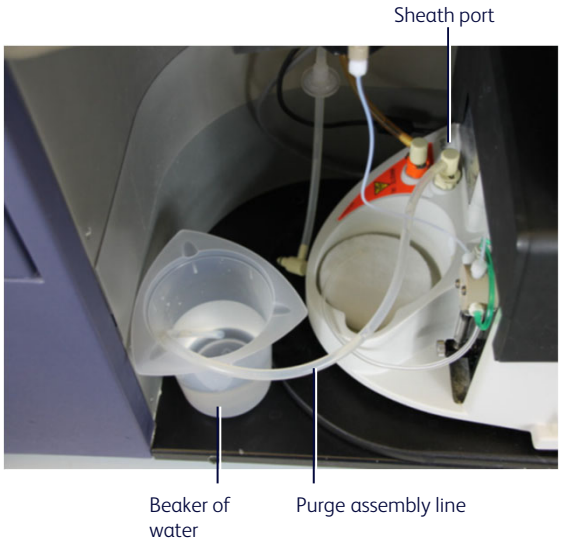
Perform these steps if you will not use the HTS within the next 24 hours. Prior to performing the steps in this section, ensure you have the purge assembly line available. The purging assembly line is included in the spares kit.

1. Press the metal button to release the sheath connector from the sheath port on the back of the HTS.
2. Connect the purging assembly line to the sheath port.



3. Put the end of the purging assembly into a 500-mL beaker containing DI water.
4. Put the safety cover on the HTS.
5. Press RUN on the cytometer fluidics control panel.
6. Select **HTS > Prime** and repeat eight times.

   Priming will replace the sheath fluid with DI water.
7. Remove the purging assembly line and reconnect the sheath line.



Turning off the system

1. Exit BD FACSDiva™ Software and shut down the computer.
2. Leave the BD FACSFlow™ Supply System on.
3. Turn off the cytometer.

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

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