Job Aid

BD FACSCanto™ 10-color and BD FACSCanto™ II Flow Cytometers: Running a performance check

This job aid provides instructions for running a performance check using manual loading on the BD FACSCanto™ 10-color and BD FACSCanto™ II Flow Cytometers. For additional information, see the *BD® Cytometer Setup and Tracking Application guide*.

Before you begin

Ensure that the cytometer has been started up according to the instrument guidelines. See your instrument's startup and shutdown job aid for more information. Make sure you allow the cytometer lasers sufficient time to warm up.

NOTE Use the same loading method (tubes or plates) when running the performance check. Using different methods might cause variations in the results and settings generated.

Materials needed

- BD FACSDiva™ CS&T Research Beads, Catalog No. 655050 (50 tests) or Catalog No. 655051 (150 tests).

Running a performance check

Preparing the beads

To prepare the beads for a performance check: In a 12 x 75-mm tube, add 1 drop of well-mixed BD FACSDiva™ CS&T Beads to 0.35 mL BD FACSFlow™ Solution.

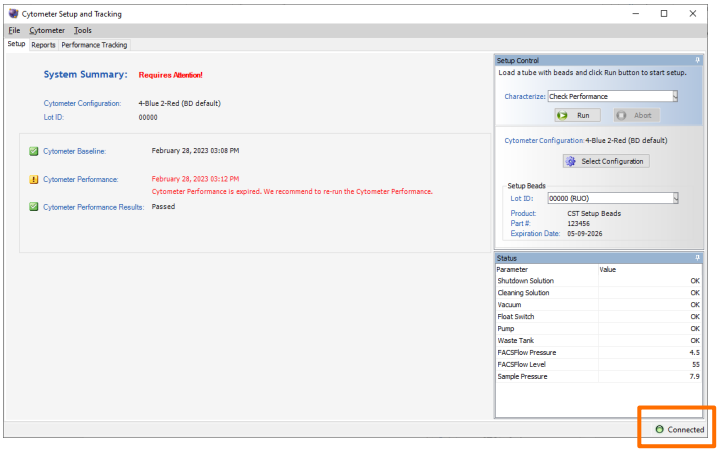
See the bead technical data insert for additional details.

Opening the BD® CS&T Application

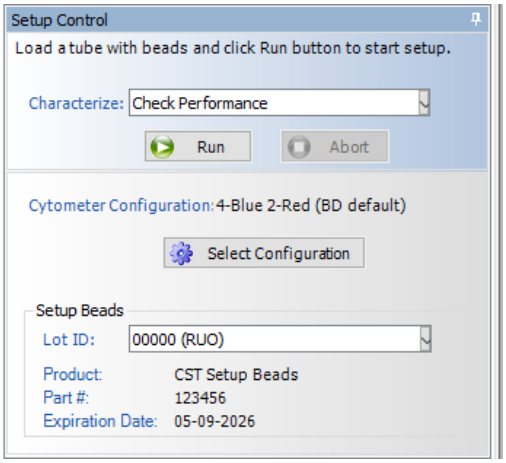
1. Select **Cytometer > CST.**

   The Cytometer Setup and Tracking workspace opens.

2. Verify that the status on the lower right corner is Connected.



Opening the BD® CS&T Application, continued



3. Verify that the cytometer configuration is correct.

   If needed, click **Select Configuration** and set a new current configuration.

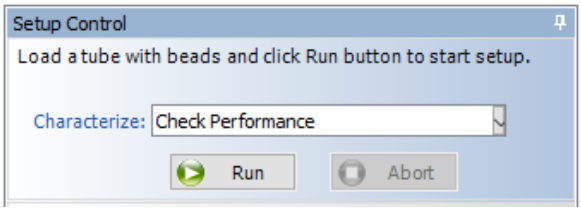
4. Verify that the bead lot ID matches the bead lot on your vial of beads. If needed, select the correct lot ID from the list.

   If your bead lot is not listed, ask your administrator to load the current bead lot file.

5. Select the **Load tube manually** checkbox, if needed.

Running the performance check

1. Vortex the prepared bead tube and install the tube on the SIP.



2. Verify the **Check Performance** is selected in the Characterize list.

3. Click **Run**.

4. Click **OK** to confirm that the tube was installed.

   The performance check takes approximately 5 minutes.

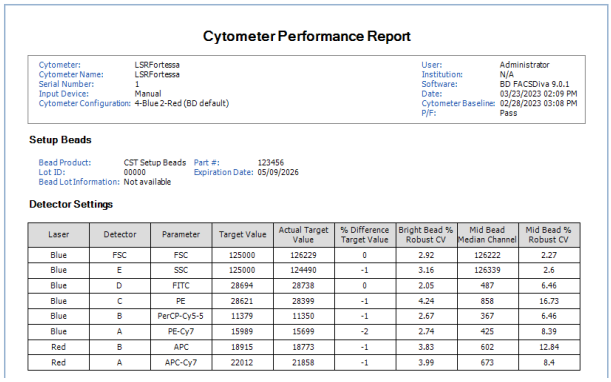
5. When the performance check completes, a dialog is displayed.

6. Remove the bead tube from the cytometer.

Viewing the performance check report

1. In the Cytometer Setup and Tracking dialog, click **View Report.**

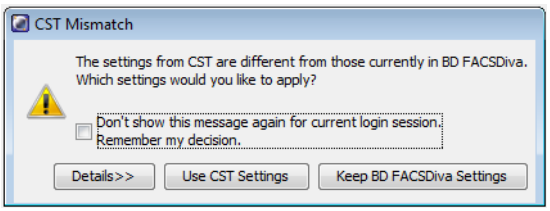
2. Verify that the cytometer performance passed.



Laser	Detector	Parameter	Target Value	Actual Target Value	% Difference Target Value	Bright Bead % Robust CV	Mid Bead Median Channel	Mid Bead % Robust CV
Blue	FSC	FSC	125000	126229	0	2.92	126222	2.27
Blue	E	SSC	125000	124490	-1	3.16	126339	2.6
Blue	D	FITC	28694	28738	0	2.05	487	6.46
Blue	C	PE	28621	28399	-1	4.24	858	16.73
Blue	B	PerCP-Cy5-5	11379	11350	-1	2.67	367	6.46
Blue	A	PE-Cy7	15989	15699	-2	2.74	425	8.39
Red	B	APC	18915	18773	-1	3.83	602	12.84
Red	A	APC-Cy7	22012	21858	-1	3.99	673	8.4

Returning to BD FACSDiva™ Software

1. Select **File > Exit** to close the workspace and reconnect to BD FACSDiva™ Software.



2. If the CST Mismatch dialog is displayed:
   a. Select the **Don't show this message again...** checkbox.
   b. Click **Use CST Settings.**

Troubleshooting

Some common issues are listed below, for more information, go to **Help > FACSDiva Help** and locate the troubleshooting table in the *BD® Cytometer Setup and Tracking Application Guide*.

No beads detected or sample rate is too low error

Problem	Solution
There are not enough beads, or the bead solution has debris.	• Make sure to vortex the tube before loading the tube on the cytometer. • Make a fresh bead suspension. Make sure to gently vortex the beads before adding them to the BD FACSFlow™ Solution and use a clean tube.
There is air, clogs or debris in the fluidics.	Check the fluidics. Make sure that the system is free of air bubbles, clogs, or debris.

Bright bead %rCV for primary channel is greater than 6%

Problem	Solution
There is air in the system.	Perform a De-gas Flow Cell procedure a couple of times.
The beads have gone bad due to exposure to light and room temperature.	Make a fresh bead suspension. Make sure that the beads have not expired or have been left out in the light.
The lasers are not ready.	Allow the cytometer lasers sufficient warm-up time. See your cytometer manual for requirements.
The flow cell is dirty.	Perform the **Clean Flow Cell** procedure. See your cytometer manual for instructions.
Cytometer alignment has changed.	Contact BD Biosciences.

PMT settings change >50 volts (or user-specified value) between performance checks

Problem	Solution
The beads have gone bad due to exposure to light and room temperature.	Make a fresh bead suspension. Make sure that the beads have not expired or have been left out in the light.
The lasers are not ready.	Allow the cytometer lasers sufficient warm-up time. See your cytometer manual for requirements.
Cytometer alignment has changed.	Contact BD Biosciences.

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