

February, 2017

Stem Cell Enumeration SOP 1: BD FACSCanto™ II Cytometer Startup Procedure

Purpose

To prepare the BD FACSCanto™ II flow cytometer for acquisition of stained samples.

Scope

This procedure applies to the clinical laboratory environment with the BD FACSCanto II flow cytometer for the purpose of CD34 enumeration using whole blood, bone marrow (fresh or thawed), cord blood (fresh or thawed), and leucopheresis (fresh or thawed) specimens. We recommend that all personnel who operate the instrument be sufficiently trained to fully perform and implement this guideline.

Equipment Required

BD FACSCanto II flow cytometer and workstation

Materials Required

Biohazard safety manual

Biohazard sharps waste container

Personal protective equipment (PPE)

- Protective gloves
- Protective eyewear
- Closed-toe shoes
- Lab coat

BD FACSTFlow™ sheath fluid (Catalog No. 342003)

BD FACST™ Clean solution (Catalog No. 340345)

BD FACST™ Shutdown solution (Catalog No. 334224)

BD FACST™ 7-color setup beads (Catalog No. 335775)

BD™ Stem Cell Control kit (Catalog No. 340991)

BD™ Stem Cell Enumeration Kit with BD Trucount™ tubes (Catalog No. 344563)

Procedure

Starting the system

1. Turn on the computer main power.
2. Log in to Windows.
3. Turn on the cytometer main power.
4. Double-click the BD FACSCanto™ clinical software icon on the desktop to start the software.
5. Log in to the software with the appropriate user and password information.



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6. Wait for the cytometer to connect. The icon in the bottom right-hand corner of the software window will turn green when the cytometer is connected.
7. Allow the cytometer to warm up for 30 minutes (20-minute minimum) before running any beads. The elapsed time will be displayed in the bottom right-hand corner of the window.

During the warm-up time for the cytometer:

1. Check the BD FACSFlow level in the **Status** window. If necessary, replace the cubitainer with a full one.
2. Check the BD FACSFlow filter on the fluidics cart for air.
 - a. If bubbles are present, loosen the cap on the filter and allow the air to escape.
 - b. Once fluid starts dripping from the filter, tighten the cap on the filter.
3. Select **Cytometer > Fluidics Startup**.
4. Click **OK** in the dialog to confirm. Fluidics startup will take about seven minutes to complete.
5. After fluidics startup has completed, select **Cytometer > Cleaning Modes > Bubble Filter Purge**. When the process is complete, click **OK**.
6. Select **Cytometer > Cleaning Modes > De-gas Flow Cell**. When the process is complete, click **OK**.
7. Repeat Step 6 to de-gas the flow cell a second time.

Preparing an optimization sample

1. Remove a vial of BD™ Stem Cell control (Low or High) from the refrigerator and allow it to stand at room temperature for 15 minutes.
2. Thoroughly mix the control sample by inverting the vial until the bottom of the vial is completely resuspended.
3. Label a Falcon® 12 x 75-mm polystyrene tube.
4. Add 20 µL of BD Stem Cell reagent to the bottom of the tube.
5. Add 100 µL of well mixed BD Stem Cell control to the tube.
6. Cap the tube, vortex gently, and incubate for 20 minutes in the dark at room temperature.
7. Add 2 mL of 1X ammonium chloride lysing solution to each tube.
8. Cap the tube, vortex gently, and incubate for 10 minutes in the dark at room temperature.
9. Immediately place tubes on wet ice and acquire within one hour of lysing.

Performing instrument QC with BD FACS 7-color setup beads

1. Open a foil pouch containing one tube of BD FACS 7-color setup beads.
2. Add the BD FACS 7-color setup bead diluent to the line on the tube.
3. Vortex the tube for two seconds to completely mix the beads and diluent.
4. Select **Cytometer > Setup > Standard Setup**.
5. Select the current bead lot ID, as listed on the sticker included in the box.
6. To enter a new bead lot using a 2D barcode reader:



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- a. Click the **Scan Barcodes** button.
- b. Scan the 2D barcode on the lot ID sticker and the information will be automatically populated into the appropriate fields.
- c. Check all affected software fields for accuracy against the setup beads label.
7. To enter a new bead lot manually:
 - a. Click the **New Lot ID** button.
 - b. Enter the lot ID and expiration date and click **OK**.
 - c. In the **Targets** tab, enter the appropriate target values printed on the sticker included in the box.
 - d. In the **Spectral Overlap Factors** tab, enter the appropriate values printed on the sticker included in the box.
 - e. Click **Finish**.
8. Click **Next**. If you changed lot ID values, click **Yes** when prompted.
9. Select **Run setup in Manual mode**, and click **Next**.
10. When prompted, load the tube of beads onto the SIT.
11. Click **OK** and wait for setup to finish.
12. Unload the beads when prompted. When the run is complete, a dialog is displayed with the message *Setup Completed Successfully*.
13. Click **View Setup Report** to print the report and review the values obtained.
14. Click **Next** to begin the optimization process.

Performing optimization for stem cell (CD34) enumeration

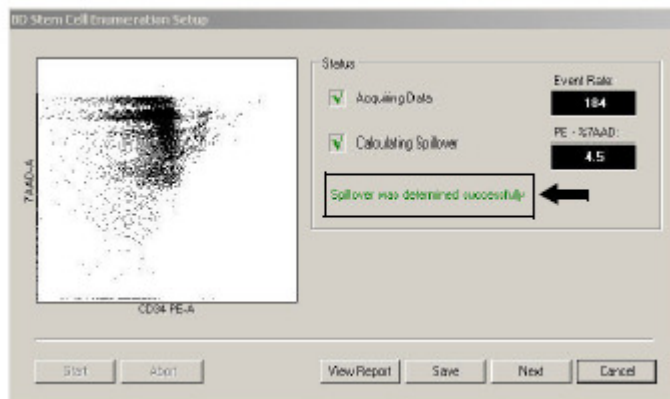
1. Select **BD Stem Cell** from the **Panel Types** menu in the Setup Optimization wizard.
2. Click **Next**.
The BD Stem Cell Enumeration Setup view opens.
3. Click **Start**.
4. Manually install the control stained with the 7-AAD (optimization) tube on the cytometer when prompted and click **OK**.
The software begins acquiring data and then calculates the spillover of 7-AAD into the PE channel.
5. Check the status message under the **Calculating Spillover** checkbox.
 - If spillover was determined successfully, go to step 6.



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- If spillover was not determined successfully, click **Cancel** and repeat steps 1 through 5.



6. Click **View Report**.
 - a. Confirm that the **Overall Result** is PASS.
 - b. Note the PE-%7AAD spectral overlap value.

A value of 0.5% to 10.5% indicates a successful setup optimization.
7. To close the report and return to the wizard, click the **Close Preview** button.
8. Click **Save**.
9. Unload the tube when prompted.
10. Click **Finish** to close the Cytometer Setup Wizard.

References

BD FACSCanto™ II Instructions for Use, document 23-12882-01.

BD FACSCanto™ Clinical Software Reference Manual, document 23-14529-00.

BD FACS™ 7-Color Setup Beads technical data sheet, document 23-12241-01, available at www.bdbiosciences.com.

BD™ Stem Cell Enumeration Kit technical data sheet, document 23-7867-04, available at www.bdbiosciences.com.

BD™ Stem Cell Control technical data sheet, document 23-4896-06, available at www.bdbiosciences.com.

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