Lymphocyte Enumeration SOP 1:
BD FACSCanto™ Cytometer Startup Procedure

Purpose

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

Scope

This procedure applies to the clinical laboratory environment with the BD FACSCanto or BD FACSCanto II flow cytometer for the purpose of lymphocyte enumeration using whole blood specimens. We recommend that all personnel who operate the instrument be sufficiently trained to fully perform and implement this guideline.

Equipment Required

BD FACSCanto or 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 FACSFlow™ sheath fluid (Catalog No. 342003)

BD FACS™ Clean solution (Catalog No. 340345)

BD FACS™ Shutdown solution (Catalog No. 334224)

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.
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.

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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.

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:
   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 **Finish** to close the Cytometer Setup Wizard.

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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 Multitest™ IMK Kit technical data sheet, document 23-3602-06, available at www.bdbiosciences.com.

BD Multitest™ 6-color TBNK technical data sheet, document 23-10834-03, available at www.bdbiosciences.com.

BD™ Multi-Check Control technical data sheet, document 23-5203-03, available at www.bdbiosciences.com.

BD™ Multi-Check CD4 Low Control technical data sheet, document 23-5208-03, available at www.bdbiosciences.com.

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