

February, 2017

Stem Cell Enumeration SOP 3: Acquisition of Stained Samples on the BD FACSCanto™ II Cytometer

Purpose

To acquire data on the BD FACSCanto™ II flow cytometer using stained whole blood, bone marrow (fresh or thawed), cord blood (fresh or thawed), and leucopheresis (fresh or thawed) specimens.

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
Vortex

Materials Required

Biohazard safety manual
Biohazard sharps waste container
Personal protective equipment (PPE)

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

Prepared immunofluorescent labeled samples for acquisition

Procedure

Before beginning this procedure, start the flow cytometer according to *Stem Cell Enumeration SOP 1: BD FACSCanto™ II Cytometer Startup Procedure*.

Creating a new worklist

1. Enter *Control* into the **Sample Name** field.
2. Enter the lot ID for the control sample and today's date in the **Sample ID** field.
3. Enter the name of the control (*BD Stem Cell High* or *BD Stem Cell Low*) in the **Case Number** field.
4. In the **Panel** column, select the **BD Stem Cell** panel.
5. Repeat steps 1 through 4 for any remaining control tubes.



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6. Enter the patient information as required for your facility.
 - **Sample ID** is a required field which can be manually entered or read from a barcode reader.
 - The **Sample Name** and **Case Number** fields are optional.
7. If the sample required dilution, add the dilution factor to the **Dilution** column for that sample. For undiluted samples, use the default value of 1.
8. In the **Panel** column, select **BD Stem Cell + 7AAD** for the patient sample.
9. Continue entering the remaining samples into the worklist.

Acquiring data

1. Push the tube guide off to the left side so that the tube can be manually installed on the SIT.
2. Vortex each tube for two seconds before manually installing the tube on the BD FACSCanto II cytometer.
3. Click **Run** in BD FACSCanto™ clinical software.
4. In the dialog that opens, enter today's date and your initials or run number. Click **Save**.
The samples will be acquired according to the specifications in the software.
5. When prompted, install the tube containing the first sample in the worklist on the cytometer and click **OK**.
6. Once the acquisition targets have been met, a dialog will prompt you to remove the current tube and install the next one in the worklist.
7. Once the worklist is complete, a dialog opens. Click **OK** and continue to data analysis.

References

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

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

BD Stem Cell Enumeration Application Guide for BD FACSCanto II Flow Cytometers, document 23-11196-01.

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