Stem Cell Enumeration SOP 2: Staining Samples with BD™ SCE Kit reagents

Purpose

To manually prepare whole blood, bone marrow (fresh or thawed), cord blood (fresh or thawed), leucopheresis specimens (fresh or thawed), and control samples using the BD™ Stem Cell Enumeration Kit reagents for the enumeration of CD34 cells using the BD FACSCanto™ II flow cytometer.

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
20-µL pipet for dispensing the reagent
100-µL electronic pipet capable of reverse pipetting
Graduated cylinder
Kimwipes® wipes
Vortex

Materials Required

Biohazard safety manual

Biohazard sharps waste container

Personal protective equipment (PPE)

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

Reagents:

- BD™ Stem Cell Enumeration Kit containing CD45 FITC/CD34 PE, 7-AAD,10X ammonium chloride lysing reagent, and BD Trucount™ tubes (Catalog No. 344563)

Deionized water

1X Dulbecco's Phosphate Buffered Saline (PBS) with 0.5% BSA (bovine serum albumin)

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Specimens:

- Peripheral whole blood
- Bone marrow (fresh or thawed)
- Cord blood (fresh or thawed)
- Leucopheresis samples (fresh or thawed)
- Specimens must be less than 24 hours old, well mixed and collected in EDTA, ACD-A, heparin, or CPD tubes.

Process controls:

- BD™ Stem Cell Control kit, containing $CD34^+$ low and $CD34^+$ high control samples (Catalog No. 340991) or equivalent

Procedure

Preparing the 1X ammonium chloride lysing solution

1. Calculate the volume of lysing solution needed for all tubes to be tested plus one spare tube.
2. Dilute the 10X concentrated ammonium chloride lysing solution 1:10 with room temperature deionized water using a graduated cylinder.
    - The prepared solution is stable for one day. Store and use at room temperature (20°C–25°C).

Preparing the samples for staining

Perform a white blood cell (WBC) count on all specimens to be evaluated. If the WBC count is greater than 40,000 cells/µL, dilute the specimen using PBS with 0.5% BSA. Use reverse pipetting to make the dilution. Record and enter the dilution factor for the calculation of the final CD34 result into the Dilution Factor column of the worklist in BD FACSCanto™ clinical software with the BD™ Stem Cell Enumeration module.

Preparing the process controls

1. Remove both vials of BD Stem Cell controls ($CD34^+$ low and $CD34^+$ high) from the refrigerator and allow to stand at room temperature for 15 minutes.
2. Thoroughly mix the control samples by rolling the vial between your hands 10 times and then inverting the vial 10 times.
3. Repeat until the bottom of the vial is completely resuspended.

Staining the cells

1. For each patient sample or process control, label the appropriate number of BD Trucount absolute counting tubes with a sample identification number.

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- When using BD Trucount tubes, verify that the bead pellet is under the metal retainer before use. If this is not the case, discard the BD Trucount tube and replace it with another. Do not transfer beads to another tube.

2. Pipette 20 µL of BD Stem Cell reagent into the bottom of each tube.
    - If using BD Trucount tubes, pipette just above the stainless steel retainer. Do not touch the pellet.
3. Pipette 20 µL of 7-AAD reagent into the bottom of each tube.
4. Pipette 100 µL of a well-mixed BD Stem Cell control or specimen into the bottom of each tube.
    - A Kimwipe wipe can be used to cover the top of the sample tube when removing the stopper to avoid splattering blood.
    - Avoid smearing blood down the side of the tube. If whole blood remains on the side of the tube, it will not be stained with the reagent and can affect results. Use a cotton tipped applicator stick dipped in deionized water to remove the blood that was smeared.
    - Accurate pipetting is critical when using BD Trucount tubes. Use the reverse pipetting technique to pipette sample onto the side of the tube just above the retainer.
    - For reverse pipetting, depress the button to the second stop. When you release the button, excess sample is drawn into the tip. Press the button to the first stop to expel a precise volume of sample. This leaves excess sample in the tip.
5. Cap the tubes and vortex to mix.
6. Incubate for 20 minutes in the dark at room temperature.
7. Add 2 mL of 1X ammonium chloride lysing solution to each tube.
8. Incubate for 10 minutes in the dark at room temperature.
9. Immediately place tubes on wet ice and acquire within one hour of lysing.

References

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

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

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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