

January, 2016

## Lymphocyte Enumeration SOP 2: 4-color Immunofluorescent Labeling of Whole Blood with BD Multitest™ IMK Reagents, Manual Preparation

### Purpose

To manually prepare whole blood and control samples using the BD Multitest™ IMK reagents for lymphocyte enumeration using the BD FACSCanto™ or BD FACSCanto™ II flow cytometer.

### 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  
20-µL pipet for dispensing the reagent  
50-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 Multitest™ CD3 FITC/CD8 PE/CD45 PerCP/CD4 APC reagent (Catalog No. 340499)
- BD Multitest™ CD3 FITC/CD16 + CD56 PE/CD45 PerCP/CD19 APC reagent (Catalog No. 340500)

BD FACS™ lysing solution (Catalog No. 349202)

BD Trucount™ absolute counting tubes (Catalog No. 340334)

Deionized water

Peripheral whole blood less than 48 hours old, well mixed and collected in EDTA tubes or equivalent



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### Process controls

- BD™ Multi-Check Whole Blood Control (Catalog No. 340912) and BD™ Multi-Check CD4 Low Control (Catalog No. 340915) or equivalent

### Procedure

#### Preparing the 1X BD FACS lysing solution

Dilute the 10X concentrated BD FACS lysing solution 1:10 with room temperature deionized water using a graduated cylinder.

- The prepared solution is stable for one month when stored in a glass container protected from light at room temperature.

#### Staining the cells

1. For each patient sample or control, label the appropriate number of 12 x 75-mm Falcon® tubes or BD Trucount absolute counting tubes with a sample identification number.
  - Use letters, such as A and B, if testing both reagents.
  - 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 Multitest CD3 FITC/CD8 PE/CD45 PerCP/CD4 APC reagent into the bottom of each tube labeled A.
  - If using BD Trucount tubes, pipette just above the stainless steel retainer. Do not touch the pellet.
3. Pipette 20 µL of BD Multitest CD3 FITC/CD16 + CD56 PE/CD45 PerCP/CD19 APC reagent into the bottom of each tube labeled B.
4. Pipette 50 µL of well mixed, anticoagulated whole blood 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 15 minutes in the dark at room temperature.
7. Add 450 µL of 1X BD FACS lysing solution to each tube.



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8. Incubate for 15 minutes in the dark at room temperature.
9. Store the samples in the dark at room temperature for up to 24 hours until they are acquired.

### References

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

BD Multitest™ IMK kit technical data sheet, document 23-3602-07, available at [www.bdbiosciences.com](http://www.bdbiosciences.com).

BD FACS™ lysing solution technical data sheet, document 23-1358-08, available at [www.bdbiosciences.com](http://www.bdbiosciences.com).

BD Trucount™ tubes technical data sheet, document 23-3483-07, available at [www.bdbiosciences.com](http://www.bdbiosciences.com).

BD™ Multi-Check Control technical data sheet, document 23-5203-03, available at [www.bdbiosciences.com](http://www.bdbiosciences.com).

BD™ Multi-Check CD4 Low Control technical data sheet, document 23-5208-03, available at [www.bdbiosciences.com](http://www.bdbiosciences.com).

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