

January, 2016

## Lymphocyte Enumeration SOP 4: Immunofluorescent Labeling of Whole Blood with BD Multitest™ 6-color TBNK Reagents or 4-color IMK Reagents, BD FACS™ SPA III Preparation

### Purpose

To prepare whole blood and control samples using the BD Multitest™ reagents and the BD FACS™ Sample Preparation Assistant II or III (SPA) 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 FACS SPA II or BD FACS SPA III instrument and workstation

### 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™ 6-color TBNK reagent (Catalog No. 644611)

Or

- 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 for 4-color assays and less than 24 hours old for 6-color assays, 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

Antifoam A Concentrate (Sigma Catalog No. A5633)

Bleach

### 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.

#### Starting the BD FACS SPA instrument

1. Remove reagents and process controls from the refrigerator and allow them to come to room temperature.
2. Open the safety cover on the instrument.
3. Close and lock the primary tube rack cage.
4. Turn on the instrument power and the computer power.
5. Empty the waste tank.
6. Add 1 L of undiluted household bleach and 500 µL of Sigma Antifoam A Concentrate to the waste tank.
7. Ensure that the waste tank fluid and electronic connectors are properly attached to the fluidics tower.
8. Fill all the bulk fluid tanks in the fluidics tower.
  - a. Empty and refill the DI water tanks with fresh water.
  - b. Empty and refill the BD FACS lyse tank if the fluid is more than one week old.
9. Reconnect the quick-release connectors to the fluid tanks and the sensors to the fluidics tower.
10. Close the safety cover.
11. Start the software and log in.
12. After the instrument has finished initializing, open the safety cover.
13. Perform the daily inspection. Check for the following:
  - Leaks or mineral deposits around the syringes.
  - Crimps in the fluidics tubing.
  - Leaks or mineral deposits around the waste port.
  - Kinks or twists in the insulation block cable.
  - Signs of probe wear.



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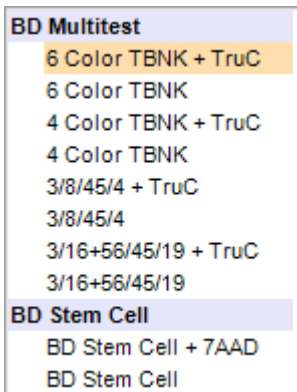
- Bubbles in the fluidics lines. If bubbles are seen, close the safety cover and perform additional priming steps.

### Preparing the secondary tubes for a run

1. For each patient sample or control, label the appropriate number of 12 x 75-mm Falcon® tubes or BD Trucount tubes with a sample identification number.
  - Use letters, such as A and B, if testing both reagents when performing 4-color testing.
  - If using BD Trucount tubes, verify that the bead pellet is under the metal retainer. If this is not the case, discard the BD Trucount tube and replace it with another. Do not transfer beads to another tube.
2. Place tubes in a green 40-tube carousel.

### Preparing the worklist

1. For the process control sample, enter *Control* into the **Sample Name** column.
2. Enter the control name in the **Sample ID** column (such as *Multi Check* or *CD4 Low*).
3. Select the appropriate panel from the panel list.



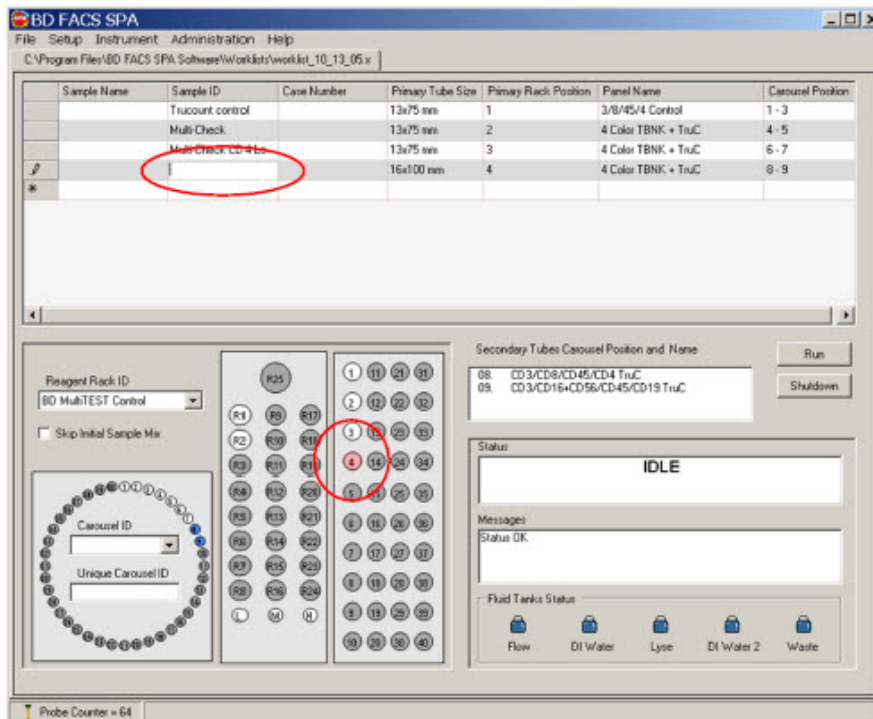
4. For patient samples, enter the sample identifier in the **Sample ID** column.
  - This information can also be added by using a barcode scanner. Place the cursor in the **Sample ID** field and scan the barcode. The information will populate the selected field.
  - If other identifiers are being used, the **Sample Name** and **Case Number** columns can be used for these entries.
5. Select the appropriate panel from the panel list for this patient.



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6. Place the specimen in the primary tube rack in the same position as the number on the worklist. Note the red colored tube location in the Primary Rack map shown below.



7. Continue adding patient and control samples as described previously.

### Setting up the reagent rack

- From the worktable area in the software, select the appropriate rack in the **Reagent Rack ID** menu.
  - For 4-color samples using both reagents, select *BD MultiTEST*.
  - For 6-color samples using the 6-color TBNK reagent, select *BD 6 Color*.
- Uncap and place the reagents into the corresponding positions on the reagent rack.
  - You can print the reagent rack map to use as an aid while loading reagents.
- Inspect the reagents to verify adequate volume and check for bubbles. If there are bubbles in the reagents, use a pipet tip to pop the bubbles before running.

### Running the worklist

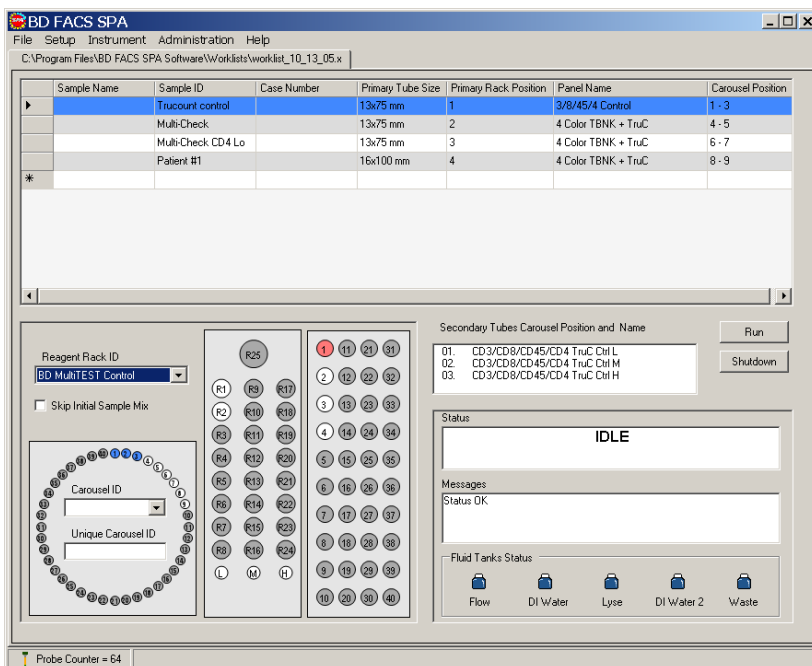
- Place the carousel containing labeled secondary tubes on the instrument worktable. Verify that the alignment pin lines up with the hole in the carousel.
- Select the carousel ID from the menu in the software.



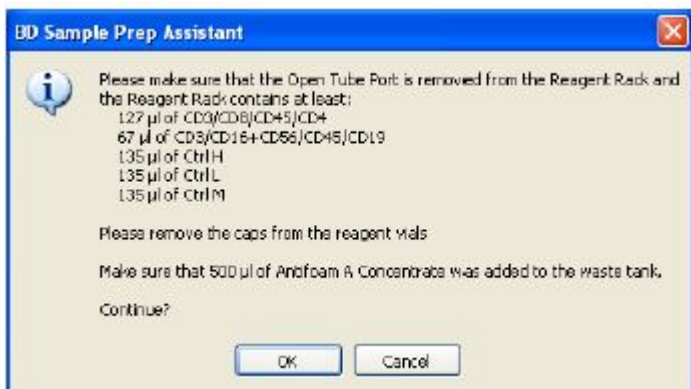
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3. Install the reagent rack onto the instrument worktable by sliding it onto the black tabs. Verify that it is installed completely within the outlined area on the worktable.
4. Close and latch the primary sample cage.
5. Close the safety cover.
6. Click **Run**.



7. A dialog opens asking you to verify reagent volumes. It also specifies the volume of each reagent needed for the run.



8. Click **OK**.
9. Save the worklist when prompted. Enter a date and your initials as the worklist name and select a location for the file. Click **OK**.

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- The instrument will mix the samples, add the reagents, add the sample, and perform all incubations.
- The status will display the name of the step and time remaining for that step while the SPA is processing samples.



- A dialog will be displayed and there will be an audible tone when the worklist is complete.



10. Click **Close** or **Print** to close the dialog.
11. Store the samples in the dark at room temperature for up to 24 hours until they are acquired. The tubes will need to be vortexed for 2 seconds before acquiring on the BD FACSCanto or BD FACSCanto II flow cytometers.

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### References

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

*BD FACS™ Sample Prep Assistant III User's Guide*, document 23-10990-00.

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

BD Multitest™ 6-color TBNK technical data sheet, document 23-10834-03, 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).

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).

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