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HLA-B27 SOP 3: Immunofluorescent Labeling of Whole Blood with BD™ HLA-B27 Reagents, BD FACS™ SPA III Preparation

Purpose

To prepare whole blood and control samples using BD™ HLA-B27 reagents and the BD FACS™ Sample Preparation Assistant II or III (SPA) for the detection of HLA-B27 antigen 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 detecting the HLA-B27 antigen 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 III instrument and workstation
12 x 75-mm Falcon® tubes or equivalent

Materials Required

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

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

Reagents:

- BD™ HLA-B27 kit, containing anti-HLA-B27 FITC/CD3 PE reagent and BD FACS™ lysing solution (Catalog No. 340183)

Distilled or deionized water, reagent grade

Phosphate buffered saline (PBS) with 0.1% sodium azide

1% paraformaldehyde in PBS

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

Process controls

- Known HLA-B27 positive and negative samples

Antifoam A Concentrate (Sigma)

Bleach



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Procedure

Preparing the 1X BD FACS lysing solution

Dilute the 10X concentrated BD FACS lysing solution 1:10 with room temperature reagent-grade 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 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 or one drop 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 distilled or deionized water.
 - b. Empty and refill the BD FACS lysing solution 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.
 - Bubbles in the fluidics lines. If bubbles are seen, close the safety cover and perform additional priming steps.



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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 with a sample identification number.
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 *HLA-B27 Positive* or *HLA B27 Negative*).
3. Select the HLA-B27 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 HLA-B27 panel from the panel list for this patient.



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- 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 following Primary Rack map.

The screenshot displays the BD FACS SPA software interface. At the top, there is a menu bar with 'File', 'Setup', 'Instrument', 'Administration', and 'Help'. Below the menu bar, the 'Prep Worklist 1' is visible. The main area contains a table with the following data:

Sample Name	Sample ID	Case Number	Primary Tube Type	Primary Rack Position	Panel Name	Carousel Position
	B27 pos control		BD Vacutainer	1	HLA-B27	1
	12345		BD Vacutainer	2	HLA-B27	2
	23456		BD Vacutainer	3	HLA-B27	3
	34567		BD Vacutainer	4	HLA-B27	4
	45678		BD Vacutainer	5	HLA-B27	5
	B27 neg control		BD Vacutainer	6	HLA-B27	6

Below the table, there is a 'Reagent Rack ID' dropdown menu and a 'Rack: 1 of 1' label. There are checkboxes for 'Skip Initial Sample Mix' and 'Incubate Offline'. A circular diagram shows the carousel positions, with 'R25' at the top. To the right, there is a 'Secondary Tubes Carousel Position and Name' section with a 'Run' button and a 'Shutdown' button. The status is 'IDLE'. The 'Fluid Tanks Status' section shows icons for Flow, DI Water, Lyse, DI Water 2, and Waste.

- Continue adding patient and control samples as described previously.

Setting up the reagent rack

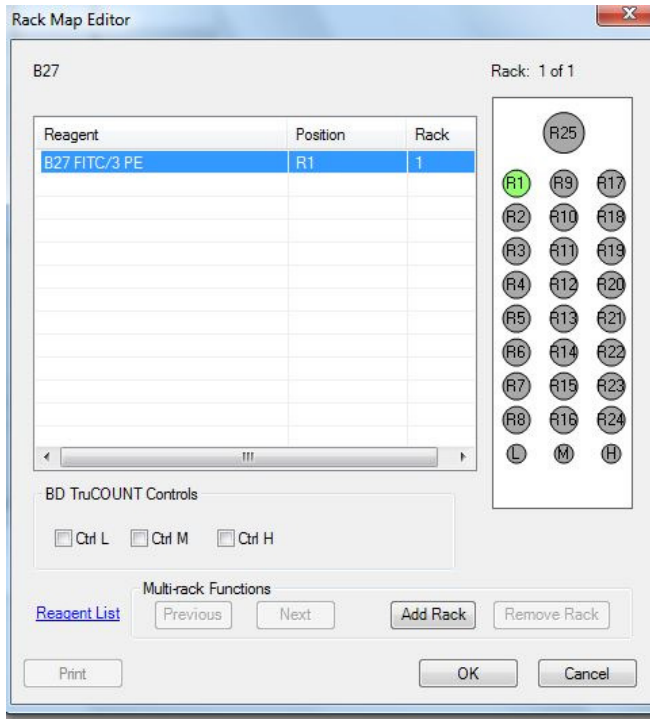
- From the worktable area in the software, select the HLA-B27 rack from the **Reagent Rack ID** menu.

The Reagent Rack must be created before the option can be selected.

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2. Uncap and place the anti-HLA-B27 FITC/CD3 PE reagent into the position on the reagent rack.



3. 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

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

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6. Click **Run**.

The screenshot displays the software interface for the BD FACS SPA III. At the top, there is a menu bar with 'File', 'Setup', 'Instrument', 'Administration', and 'Help'. Below the menu bar, the 'Prep Worklist 1' is visible. The main area contains a table with the following data:

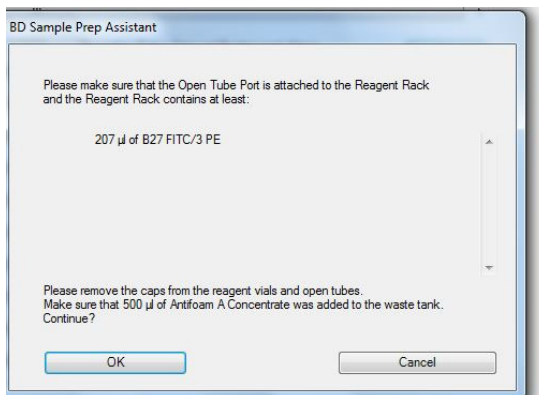
Sample Name	Sample ID	Case Number	Primary Tube Type	Primary Rack Position	Panel Name	Carousel Position
B27 pos control			BD Vacutainer	1	HLA-B27	1
	12345		BD Vacutainer	2	HLA-B27	2
	23456		BD Vacutainer	3	HLA-B27	3
	34567		BD Vacutainer	4	HLA-B27	4
	45678		BD Vacutainer	5	HLA-B27	5
B27 neg control			BD Vacutainer	6	HLA-B27	6

Below the table, there are several control panels:

- Reagent Rack ID:** A dropdown menu set to 'B27'. Below it, 'Rack: 1 of 1' is shown. There are checkboxes for 'Skip Initial Sample Mix' (unchecked) and 'Incubate Offline' (checked). A circular diagram of the reagent rack is shown with 'Carousel ID' set to '1' and a 'Unique Carousel ID' field.
- Secondary Tubes Carousel Position and Name:** A text box containing '01. Untitled-Tube'. There are 'Run' and 'Shutdown' buttons.
- Status:** A large text box displaying 'IDLE'.
- Messages:** A text box displaying 'Status OK'.
- Fluid Tanks Status:** A row of five icons representing 'Flow', 'DI Water', 'Lyse', 'DI Water 2', and 'Waste'.

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

The required amount of reagent will depend on the number of samples to be stained in the run.



8. Click **OK**.



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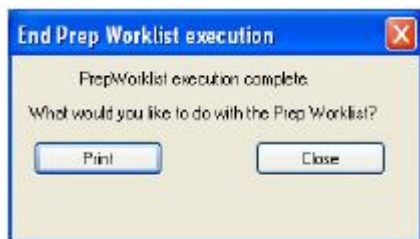
9. Save the worklist when prompted.
 - a. Enter a date and your initials as the worklist name and select a location for the file.
 - b. If the worklist is being imported into BD FACSCanto™ clinical software, select the appropriate location.
 - c. Click **OK**.

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. Immediately after incubation, centrifuge tubes at 300g for 5 minutes at room temperature (20 °C-25 °C).
12. Aspirate the supernatant, leaving approximately 50 µL of residual fluid in the tube to avoid disturbing the pellet.
13. Vortex tubes thoroughly at low speed to resuspend the cell pellet in the residual volume. Add 2 mL of BD CellWASH™ solution or PBS with 0.1% sodium azide to each tube.
14. Vortex thoroughly at low speed for 3 seconds and centrifuge at 200g for 5 minutes at room temperature (20 °C-25 °C).
15. Aspirate the supernatant, leaving approximately 50 µL of residual fluid in the tube to avoid disturbing the pellet.
16. Vortex tubes thoroughly at low speed to resuspend the cell pellet in the residual volume. Add 0.25 mL of 1% paraformaldehyde in PBS to each tube, and vortex thoroughly at low speed for 3 seconds. Make sure cells are well mixed with the fixing solution.

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17. Samples are now ready to be analyzed on the flow cytometer. Observe the following guidelines:
- Cap or cover and store the prepared tubes at 2°C–8°C in the dark until flow cytometric analysis.
 - Analyze the fixed cells within 24 hours of staining.
 - Vortex the cells thoroughly at low speed to reduce aggregation before analyzing on the flow cytometer.

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™ HLA-B27 Kit technical data sheet, document 23-2563-13 available at www.bdbiosciences.com.

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