HLA B27 SOP 2: Immunofluorescent Labeling of Whole Blood with BD™ HLA-B27 Reagents, Manual Preparation

Purpose

To manually prepare whole blood and control samples using BD™ HLA-B27 reagents for detecting the 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 FACSCanto II flow cytometer and workstation
12 x 75-mm Falcon® tubes or equivalent
30-µL pipet for dispensing the reagent
50-µL electronic pipet
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™ 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 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 positive and negative HLA-B27 samples

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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.

Staining the cells

1. For each patient sample or control, label the appropriate number of 12 x 75-mm Falcon tubes with a sample identification number.
2. Pipette 30 µL of anti-HLA-B27 FITC/CD3 PE into the bottom of each tube.
3. 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.
4. Cap the tubes and vortex on low speed for 3 seconds to mix.
5. Incubate for 15 to 20 minutes in the dark at room temperature (20°C–25°C).
6. Add 2 mL of room temperature (20°C–25°C) 1X BD FACS lysing solution to each tube. Immediately vortex at low speed for 3 seconds.
7. Incubate for 10 to 12 minutes in the dark at room temperature (20°C–25°C). Do not exceed 12 minutes incubation.
8. Immediately after incubation, centrifuge tubes at 300*g* for 5 minutes at room temperature (20°C–25°C).
9. Aspirate the supernatant, leaving approximately 50 µL of residual fluid in the tube to avoid disturbing the pellet.
10. Vortex tubes throughly at low speed to resuspend the cell pellet in the residual volume. Add 2 mL of 1X PBS with 0.1% sodium azide to each tube.
11. Vortex thoroughly at low speed for 3 seconds and centrifuge at 200*g* 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 throughly 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 cellls are well mixed with the fixing solution.

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

23-19154-00