



PRODUCT INSERT

HLA-B27 FLUORESCHEIN ISOTHIOCYANATE (FITC)



CONJUGATED MONOCLONAL ANTIBODY

Catalog #B27F50X (10 µl/test)



For In Vitro Diagnostic Use.



INTENDED USE

A qualitative, whole blood procedure for direct immunofluorescence staining of HLA-B27 surface antigens with analysis by flow cytometry.

SUMMARY AND EXPLANATION

HLA-B27 FITC Conjugated Monoclonal Antibody reacts specifically to the B27 Human Leukocyte Antigen. This reagent is to be used in immuno-staining of human lymphocytes in whole blood. The fluorescence intensity of lymphocytes can be analyzed on a flow cytometer after erythrocytes are lysed and separated. HLA B27 has been found to be highly associated with ankylosing spondylitis. Testing for the presence of HLA-B27 antigen in a patient is used to confirm the diagnosis of ankylosing spondylitis.

PRINCIPLE(S)

HLA-B27 FITC Conjugated Monoclonal Antibody detects cells bearing the B27 antigen on their membranes. Whole blood is first stained with the HLA-B27 FITC Conjugated Monoclonal Antibody, followed by lysis of red blood cells and fixation of white blood cells with formaldehyde. Flow cytometric analysis is then performed on white blood cells after removal of debris.

REAGENTS

A. Identification

- 1. Specificity: HLA-B27
2. Clone: FD705-9E1E10 is derived from hybridization of mouse P3X63Ag8.653 myeloma cells with spleen cells from CB6F1 mouse immunized with an HLA-B27 positive human cell line.
3. IgG Chain Composition: Mouse IgG2b heavy chain and kappa light chain.



B. Warning or Caution



- 1. For In Vitro Diagnostic Use.
2. Warning: All blood products should be treated as potentially infectious.
3. Warning: This reagent contains 0.1% sodium azide, which under acidic conditions yields hydrazonic acid, an extremely toxic compound.
4. Warning: Formaldehyde is toxic and allergenic and is a suspected carcinogen.
5. Caution: Do not use the reagent if precipitate is observed.
6. Refer to the Material Safety Data Sheet for detailed information.

C. Instructions for Use

See Directions for Use.



D. Storage Instructions

Store in the dark at 2-5° C. Use before expiration date printed on the package.

- E. Instability Indications  
Do not use if precipitate is observed.

## **INSTRUMENT REQUIREMENTS**

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FACScan or equivalent.

## **SPECIMEN COLLECTION AND PREPARATION**

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The blood specimen should be collected in an EDTA (K3) Vacutainer<sup>®</sup> blood collection tube and must be analyzed within three days. However, ACD or sodium heparin may also be used.

## **PROCEDURE**

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- A. Materials Provided  
HLA-B27 FITC conjugated monoclonal antibody.
- B. Materials Required, But Not Provided
- FACS<sup>®</sup> Brand Lysing Solution 10X Concentrate (Becton Dickinson, Order #92-0002 or equivalent).
  - Phosphate Buffered Saline (PBS) (Irvine Scientific, Catalog #9242 or equivalent)
  - Fixing solution : PBS with 0.5% formaldehyde; add 1.35 ml 37% formaldehyde to 100 ml PBS.
  - Negative control: mouse IgG2b FITC conjugated monoclonal antibody. (OLI Cat. #G2BF50X)
  - Positive control: HLA Class I FITC conjugated monoclonal antibody. (OLI Cat. #HLA1F50X)
- C. Step-by-step procedure.  
See Directions For Use.

## **DIRECTIONS FOR USE**

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1. To avoid volume loss, centrifuge vial for a few seconds in a microcentrifuge before opening (liquid may accumulate in cap during shipment).
2. Pipette 100 µl of whole blood sample into a 12 x 75 mm tube.
3. Pipette 10 µl HLA-B27 FITC conjugated monoclonal antibody into the tube and mix well with gentle vortex.
4. Incubate the tube in the dark at 2-5° C for 15 minutes with gentle rotation.
5. Dilute 10X lysing solution 1:10 with glass-distilled water. Add 3 ml of 1X lysing buffer to the tube.
6. Vortex and incubate the tube in the dark at room temperature for 10 minutes.
7. Centrifuge the tube at 300 g for 5 minutes.
8. Aspirate the supernatant.
9. Resuspend the pellet in 2 ml of PBS and vortex the tube.
10. Centrifuge the tube at 300 g for 5 minutes.
11. Aspirate the supernatant.
12. Resuspend the pellet in 0.5 ml of fixing solution. The cells are ready for immediate flow cytometric analysis, or they can be stored in the dark at 2-5° C for up to 24 hours before being analyzed.

## **LIMITATIONS OF THE PROCEDURE**

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- A. EDTA is the anticoagulant of choice. However, ACD and sodium heparin can also be used.
- B. Sterile blood samples should be stored at room temperature and analyzed within 3 days of collection.
- C. The volume of reagent recommended is based on studies of normal human blood.
- D. Laboratories using a procedure and/or instrument other than those recommended here may need to adjust the volume of reagent needed for each sample to obtain best results.

## **DATA COLLECTION**

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- A. Align and quality control the flow cytometer daily, according to the manufacturer's recommended start-up procedure.
- B. For first-time users, at least 5 known HLA-B27 positive and 10 known HLA-B27 negative samples should be tested on the flow cytometer to establish the range of gated peak channel and mean channel for B27 positive and negative phenotypes.
- C. HLA-B27 positive and negative controls should be run daily before sample analysis is performed. If the controls are out of range, re-align and quality control (QC) the flow cytometer. The known positive control can be from a freshly-drawn donor or an aliquot from a pool of cryopreserved lymphocytes that are thawed and stained along with the test samples.

More frequent QC is advised when a large number of samples is being analyzed. It is recommended that the fluorescence of negative populations be adjusted to between channels 10 and 20.

- D. Collect a green fluorescence for 5,000–10,000 events for each sample.
- E. Gate around the lymphocyte region and obtain peak channel and mean (Fig. 1, 2, and 3).
- F. Assign HLA-B27 phenotype based on the established criteria.

### EXPECTED VALUES

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- A. HLA-B27 positive samples will have a gated mean channel of >70 and HLA-B27 negative samples will have a gated mean channel of <40, based on a study performed in our laboratory using a FACS brand flow cytometer. The expected values may vary, depending upon each laboratory's testing conditions and calibration of the flow cytometer. Each laboratory should establish the normal ranges of HLA-B27 positive cells and HLA-B27 negative cells under its own testing conditions for each batch of samples.
- B. Most of the samples used to obtain the gated mean channel values were obtained from Caucasian donors. The range of values may be different for samples from other races.
- C. Most B27 subtypes (B27-01, 02, 03, 04, 05, 06, 07, and 09) tested showed positive reactions; however, B2708 showed negative reactions. Unlike the most B27 subtypes, which behave serologically like Bw4, B2708 is Bw6 associated.
- D. Certain samples with B44, B57 may generate higher background. Therefore, a few samples with those typings should be included for the initial establishment of positive and negative ranges.
- E. Individual lots of B27 FITC may have different mean channel shifts. Therefore, the positive/negative cut-off may have to be adjusted according to the performance of each individual lot.

### BIBLIOGRAPHY

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- 1. *HLA and Disease Associations*, Eds. Tiwari, JL and Terasaki, PI. Springer-Verlag, New York, 1985.
- 2. Pei, R, et al. A monospecific HLA-B27 fluorescein isothiocyanate-conjugated monoclonal antibody for rapid, simple and accurate HLA-B27 typing. *Tissue Antigens*, 41: 200-203, 1993.
- 3. NCCLS Tentative Standard. "Leukocyte Differential Counting." Publication Number H20-T, NCCLS Vol. 4, No. 11, 1984.
- 4. Ward, AM and Nikaein, A. Comparison of monoclonal antibodies for flow cytometric analysis of HLA-B27 antigen. *Cytometry (Communications in Clinical Cytometry)* 22:65-69, 1995.

### ABBREVIATIONS USED IN THIS DOCUMENT

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<b>ACD</b>	Acid Citrate Dextrose
<b>CV</b>	Coefficient of Variation
<b>EDTA</b>	Ethylenediaminetetracetic Acid
<b>FITC</b>	Fluorescein Isothiocyanate
<b>FSC</b>	Forward Scatter
<b>HLA</b>	Human Leukocyte Antigen
<b>PkChl</b>	Peak Channel
<b>SD</b>	Standard Deviation
<b>SSC</b>	Side Scatter

### TRADEMARKS USED IN THIS DOCUMENT

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FACS	Registered trademark of Becton-Dickinson.
Vacutainer	Registered trademark of Becton-Dickinson.

**EC REP** EUROPEAN AUTHORIZED REPRESENTATIVE

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# REFERENCE HISTOGRAMS AND DOT PLOTS

## B27+ and B27- Sample Histograms (Gated)

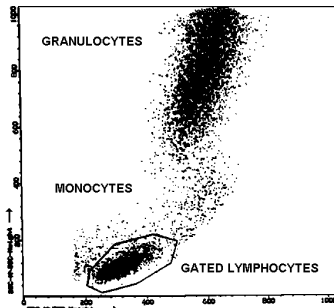


Figure 1. Dot Plot (FSC vs. SSC) represents whole lysed blood (lymphocyte gated).

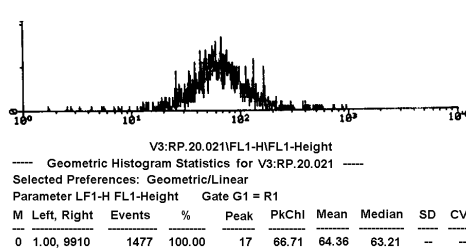


Figure 2. B27+ sample FL-1 histogram and statistics (gated).

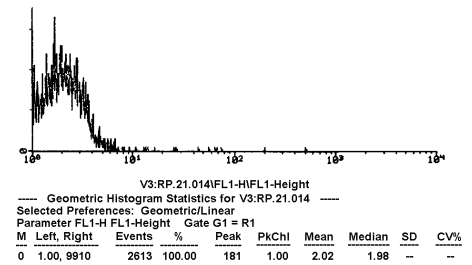


Figure 3. B27- sample FL-1 histogram and statistics (gated).

### Sample Dot Plots for Lysed and Unlysed Blood (demonstrates what can occur if the sample preparation is poor)

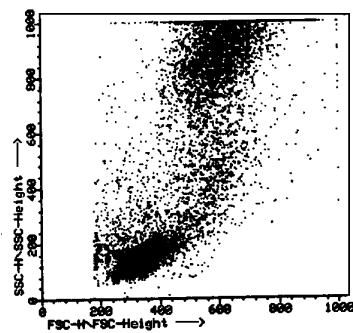


Figure 4a. Lysed blood

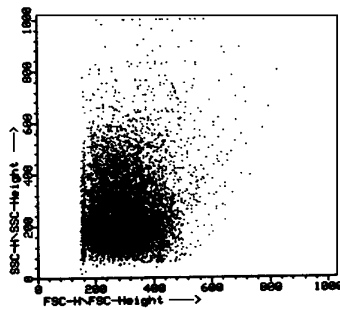


Figure 4b. Unlysed blood

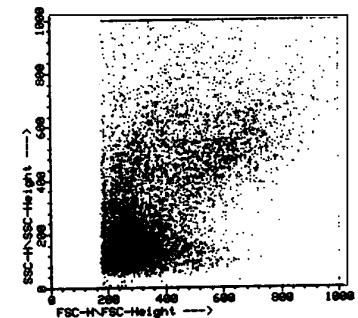


Figure 4c. Partially lysed blood

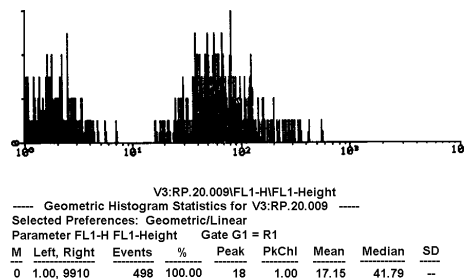


Figure 5. Partially lysed B27+ sample FL-1 histogram and statistics (gated).

## TROUBLESHOOTING

Problem	Cause	Solution
1. Abnormal dot plot pattern	<p>a. FACS parameters are not correctly set.</p> <p>b. The whole blood was not lysed well.</p>	<p>a. Make sure the FACS parameter settings are correct. This can be done by using any fresh whole blood.</p> <p>b. Check the lysing buffer and lysing conditions. If the correct lysis buffer and conditions were used:</p> <ul style="list-style-type: none"> <li>▪ Take the unlysed sample, spin down the cells, and repeat the lysing procedure.</li> <li>▪ Increase the amount of lysing buffer to 4 ml.</li> <li>▪ Increase the lysing incubation temperature to 37° C.</li> <li>▪ Increase the lysing incubation time to 20 minutes</li> </ul> <p>(Note: The above steps may be tried either one</p>

<b>Problem</b>	<b>Cause</b>	<b>Solution</b>
		at a time or together for maximum RBC lysis.

<b>Problem</b>	<b>Cause</b>	<b>Solution</b>
2. Low signal of B27 positive samples	<ul style="list-style-type: none"> <li>a. The flow cytometer needs alignment.</li> <li>b. The whole blood may not be lysed well.</li> <li>c. The blood was not mixed well with the antibody.</li> </ul>	<ul style="list-style-type: none"> <li>a. Realign the instrument.</li> <li>b. Check the dot plot of the sample. If it looks abnormal or the histogram shows two peaks, lyse the sample again. Refer to 1b above in this table.</li> <li>c. Make sure the blood is mixed well with the antibody.</li> </ul>
3. High background of the B27 samples.	<ul style="list-style-type: none"> <li>a. The whole blood may not be lysed well.</li> <li>b. The antibody incubation may be longer than 15 minutes.</li> <li>c. The FITC-antibody concentration may be higher than recommended</li> <li>d. Higher than recommended amount of antibody was used for the test.</li> </ul>	<ul style="list-style-type: none"> <li>a. See solution 1b above.</li> <li>b. Incubate for 15 minutes.</li> <li>c. Check FITC antibody concentration.</li> <li>d. Check amount of antibody used.</li> </ul>