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P R O D U C T I N S E R T

SIMULTAG® HLA-B27 FLUORESCEIN ISOTHIOCYANATE (FITC)/ CD3 R-PHYCOERYTHRIN (PE) MONOCLONAL ANTIBODY

REF

Catalog # 27FC3P50

IVD

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.[1] CD3 PE-conjugated monoclonal antibody is used to identify human T-lymphocytes, thus facilitating the analysis of HLA-B27 on the T-cell population.

PRINCIPLE

HLA-B27 FITC/CD3-PE conjugated monoclonal antibody detects cells bearing the B27 antigen on their membranes. Whole blood is first stained with the HLA-B27 FITC/CD3 PE-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 CD3-bearing T-lymphocytes after removal of debris.[2]

REAGENTS

A. Identification

1. Specificity: HLA-B27
   Clone: FD705-9E1E10 is derived from hybridization of mouse P3X63Ag8.653 myeloma cells with spleen cells from a CB6F1 mouse immunized with an HLA-B27 positive human cell line.[2] The antibody is purified from ascites fluids by protein G affinity chromatography.
   Ig Chain Composition: Mouse IgG2b heavy chain and kappa light chain.
2. Specificity: Human CD3
   Clone: B-B12 is derived from hybridization of mouse SP2/p myeloma cells with spleen cells from a Balt/C mouse immunized with cells of a human T-cell leukemia.[3] The antibody is purified from ascites fluids by protein G affinity chromatography.
   Ig Chain Composition: Mouse IgG1 heavy chain and kappa light chain.
3. Product Information
   - **HLA-B27 Antibody**
     Concentration: 0.1 mg/ml
     F:P Ratio: 6.0-7.0
   - **CD3 Antibody**
     Concentration: 0.1 mg/ml
     A566: A280 Ratio: 0.80
   - **Total Volume**
     500 µl (50 tests)
   - **Measurement of Activity**
     Follow the testing procedure suggested using HLA-B27 positive cells.
   - **Specificity Analysis**
     Immunofluorescent flow cytometric testing

B. Warnings or Cautions

1. For In Vitro Diagnostic Use.
2. **Warning:** All blood products should be treated as potentially infectious. Source material from which this product was derived was found negative when tested in accordance with current FDA required tests. No known test methods can offer assurance that products derived from human blood will not transmit infectious agents.
3. **Warning:** This reagent contains 0.1% sodium azide, which under acidic conditions yields hydrazonic acid, an extremely toxic compound. Reagents containing sodium azide should be diluted in running water prior to being discarded. These conditions are recommended to avoid deposits in plumbing where explosive conditions may develop.
4. **Warning:** Formaldehyde is toxic and allergenic, and is a suspected carcinogen. Formaldehyde is listed as a carcinogen in California. Avoid contact with eyes, skin, and clothing.
5. Refer to the Material Safety Data Sheet for detailed information.

C. Instructions for Use

See "Directions for Use" on next page.

D. Storage Instructions

This reagent is sensitive to light and must be stored in the dark. Store reagents at temperature indicated on package. Use before printed expiration date.

E. Purification or Treatment Required for Use

EDTA is the anticoagulant of choice. However ACD and sodium heparin can also be used.

F. Instability Indications

Do not use reagent if precipitate is observed.

INSTRUMENT REQUIREMENTS

FACScan® or equivalent flow cytometer

SPECIMEN COLLECTION AND PREPARATION

Blood sample requirements:

- The preferred tube for blood specimen collection is an EDTA (K3) Vacutainer® blood collection tube; however, ACD or sodium heparin may also be used.
- Sterile blood samples should be stored at room temperature (20 – 25° C) and analyzed within three days of collection.

PROCEDURE

A. Materials Provided

HLA-B27 FITC conjugated monoclonal antibody

B. Materials Required, But Not Provided

1. FACS® Brand Lysing Solution 10X Concentrate (Becton Dickinson, Order #92-0002) or equivalent.
2. Phosphate Buffered Saline (PBS) (Irvine Scientific, Catalog #9242) or equivalent.
3. Fixing solution: PBS with 0.5% formaldehyde; add 1.35 ml of 37% formaldehyde to 100 ml PBS.

C. Step-by-step procedure.

See "Directions For Use" in box (below or above).

DIRECTIONS FOR USE

A. To avoid volume loss, centrifuge vial for a few seconds in a microcentrifuge before opening. (Liquid may accumulate in cap during shipment.)

B. Pipet 100 µl whole blood sample into a 12 x 75 mm tube.

C. Pipet 10 µl Simultag® HLA-B27 FITC/CD3PE Monoclonal Antibody into the tube and mix well with gentle vortex.

D. Incubate tube in the dark at 2 – 5 ° C for 15 minutes with gentle rotation.

E. Dilute 10X lysing solution 1:10 with glass-distilled water. Add 3 ml 1X lysing buffer to the tube.

F. Vortex and incubate the tube in the dark at room temperature for 10 minutes.

G. Centrifuge the tube at 300 g for 5 minutes.

H. Aspirate the supernatant.

I. Resuspend the pellet in 2 ml PBS and vortex the tube.

J. Centrifuge the tube at 300 g for 5 minutes.

K. Aspirate the supernatant.

L. Resuspend the pellet in 0.5 ml 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.

RESULTS

Data Analysis

A. Align and quality control (QC) the flow cytometer daily according to the manufacturer's recommended procedure.

B. First time users should test at least 5 known HLAB27 positive and 10 known HLA-B27 negative samples 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 QC the flow cytometer. The known positive control can be from a freshly drawn donor or aliquoted from a pool of cryo-preserved lymphocytes, which are thawed and stained along with the test samples. More frequent QC is advised when large numbers of samples are analyzed. We recommend adjustment of the fluorescence of the negative populations to fall between channels 10 and 20.

D. Collect a green fluorescence for 5,000 – 10,000 events for each sample.

E. Obtain a CD3 positive cell population by gating R2 from the FL2 histogram of gated lymphocytes (Figures 1 and 2).

F. Obtain the FL1 peak channel and the mean of the CD3 positive lymphocytes—gated R2 (Figures 3 and 4).

G. Assign HLA-B27 phenotype based on the established criteria.

LIMITATIONS OF THE PROCEDURE

- The volume of reagents recommended is based on studies of normal human blood.
- Laboratories using a procedure and/or instrument other than those indicated here may need to adjust the volume of reagents needed for each sample to obtain best results.

EXPECTED VALUES

A. HLA-B27 positive samples will have a gated mean channel of >66 and HLA-B27 negative samples will have a gated mean channel of <33, based on a study performed in our laboratory using a FACS brand flow cytometer. The expected values may vary depending on each laboratory's testing conditions and calibration of the flow cytometer. Each laboratory should establish the normal range so 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 gated mean channel values were obtained from Caucasian donors. The range of values may be different for samples from other races.

SPECIFIC PERFORMANCE CHARACTERISTICS

A. Different flow cytometers may obtain different mean channel values.

B. The HLA-B27 monoclonal antibody reacts with B2701, B2702, B2703, B2704, B2705, B2706, B2707, and B2709 subtypes. It does **not react** well with B2708. Another supplementary clone, B7,27 (OLI Cat.# FH1453), does react to B2708, and it can be used to clarify any ambiguous typings.

TROUBLESHOOTING

Description of Problem	Causes	Solutions
1. Abnormal dot plot pattern	a. FACS parameters are not correctly set.	a. Make sure the FACS parameter settings are correct. This can be done by using any fresh whole blood.
	b. The whole blood was not lysed well. (See Figures 5a, 5b, and 5c.)	b. Check the lysing buffer and lysing conditions. If the correct lysis buffer and conditions were used: • For the unlysed sample, spin down the cells and repeat the lysing procedure. • Increase the amount of lysing buffer to 4 ml. • Increase the lysing incubation temperature to 37° C. • Increase the lysing incubation time to 20 minutes. (Note: The above steps may be tried either one at a time or together for maximum RBC lysis.
2. Low signal of the B27 positive samples	a. The flow cytometer needs alignment.	a. Recheck the positive and negative controls to make sure they fall within the correct range.
	b. The whole blood may not be lysed well.	b. Check the dot plot of the sample. If it looks abnormal or the histogram shows two peaks, lyse the sample again. Refer to #1.b in this table.
	c. The blood was not mixed well with the antibody.	c. Make sure the blood is mixed well with the antibody.
3. High background of the B27 samples	a. The whole blood may not be lysed well.	a. Refer to #1.b in this table.
	b. The antibody incubation may be longer than 15 minutes.	b. Incubate for the recommended time.
	c. The FITC-antibody concentration may be higher than recommended.	c. Use recommended FITC-antibody concentration.

BIBLIOGRAPHY

1. HLA and Disease Associations. Eds. Jawahar L. Tiwari and Paul I. Terasaki, (1985) Springer-Verlag, New York.
2. Rui Pei, 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. Clevers et al. Ann. Rev. Immunol, 1988: 6: 629.

TRADEMARKS USED IN THIS DOCUMENT

EC REP EUROPEAN AUTHORIZED REPRESENTATIVE

MDSS GmbH, Burckhardstrasse 1, D-30163, Hannover, Germany

CE 0197

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

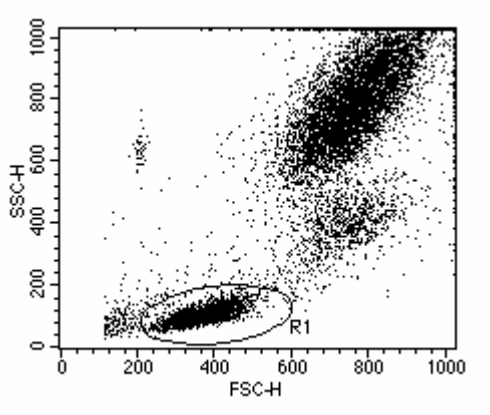


Figure 2: FL2 histogram of gated lymphocytes. R2 gates for CD3 positive lymphocytes.

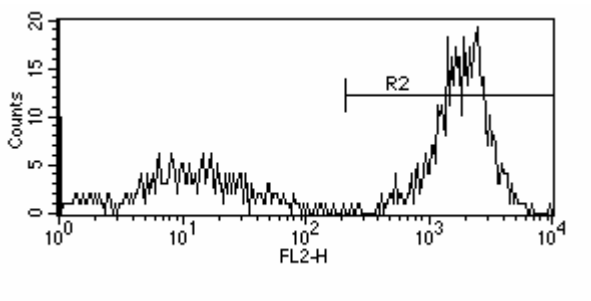


Figure 3: FL1 histogram and statistics on gated CD3 positive lymphocytes for B27 negative sample.

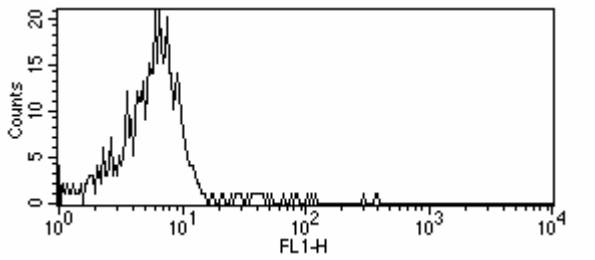
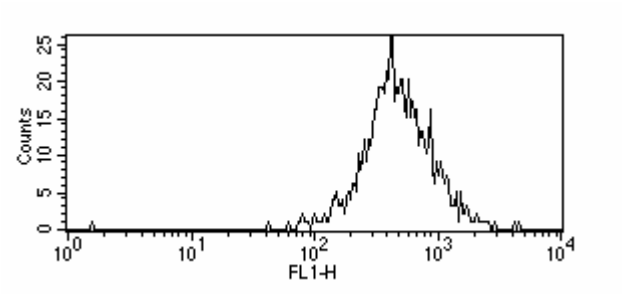


Figure 4: FL1 histogram and statistics on gated CD3 lymphocytes



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

Figure 5a: Lysed Blood

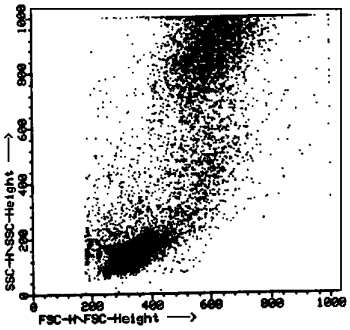


Figure 5b: Unlysed Blood

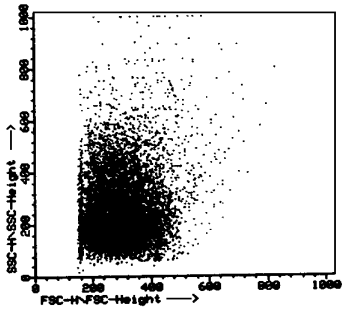
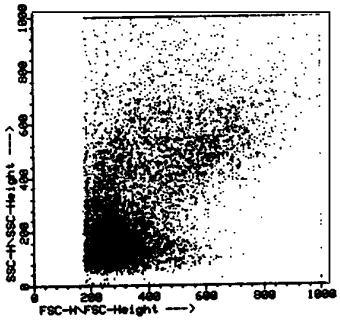
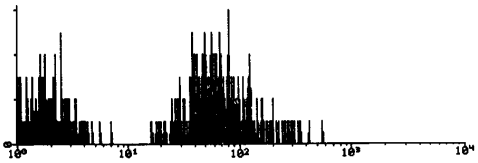


Figure 5c: Partially lysed blood.





V3:RP.20.009\FL1-H\FL1-Height
----- Geometric Histogram Statistics for V3:RP.20.009 -----
Selected Preferences: Geometric/Linear
Parameter FL1-H FL1-Height Gate G1 = R1

M	Left, Right	Events	%	Peak	PkChl	Mean	Median	SD	CV %
0	1.00, 9910	498	100.00	18	1.00	17.15	41.79	--	--

Figure 6: Partially lysed blood. Histogram and statistics (gated) for B27 sample.

REVISION HISTORY

Revision	Date	Revision Description
1B	*2005/09*	*Delete expired R-Phycoerythrin patents and update template.*