One Lambda, Inc.
21001 Kittridge Street, Canoga Park, CA 91303-2801 Tel: (818) 702-0042 Fax: (818) 702-6904 WEB:www.onelambda.com

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

HLA Class I Fluorescein Isothiocyanate (FITC) Conjugated Monoclonal Antibody

REF ***Catalog #HLA1F50X***

IVD ***For In Vitro Diagnostic Use.***

INTENDED USE

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

SUMMARY AND EXPLANATION

HLA Class I FITC Conjugated Monoclonal Antibody reacts specifically to the Class I Human Leukocyte Antigen. This reagent is to be used in immuno-staining of human lymphocytes in whole blood. The fluorescent intensity of lymphocytes can be analyzed on a flow cytometer after erythrocytes are lysed and removed.

PRINCIPLE

HLA Class I FITC Conjugated Monoclonal Antibody detects cells bearing the Class I antigen on their membranes. Whole blood is first stained with the HLA Class I 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 Class I
2. **Ig Chain Composition:** Mouse IgG1 heavy chain and kappa light chain.
3. **F:P Ratio:** 6.0 – 7.0
4. **Measurement of Activity:** Follow the testing procedure suggested using HLA Class I positive cells.
5. **Specificity Analysis:** Immunofluorescent flow cytometry testing
6. **Suggested Working Dilution:** Use 2 µl for 100 µl whole blood in the flow cytometry test

B. Warning or Caution

1. **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. Refer to the Material Safety Data Sheet for detailed information.
2. **Warning**: Formaldehyde is toxic, allergenic and is a suspected carcinogen. Formaldehyde is listed as a carcinogen in California. Avoid contact with eyes, skin and clothing. Refer to the Material Safety Data Sheet for detailed information.
3. 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 known human blood will not transmit infectious agents.

C. Instructions for Use

See **DIRECTIONS FOR USE** on page 2.

D. Storage Instructions

Store reagents at temperature indicated on package. The reagent is stable for the period shown on the label when stored in the dark at 2-5° C.

E. Instability Indications

Do not use the reagent if precipitates are observed.

INSTRUMENT REQUIREMENTS

FACScan® or equivalent flow cytometer.

SPECIMEN COLLECTION AND PREPARATION

The blood specimen should be collected in an EDTA (K_3) Vacutainer blood collection tube and analyzed within three days. ACD or sodium heparin may also be used.

PROCEDURE

A. Materials Provided

1 - 100 µl vial HLA Class I Fluorescein Isothiocyanate (FITC) Conjugated Monoclonal Antibody (50 tests)

Note: *The volumes provided are slightly more than the amount required for testing. This is to account for inadvertent loss which may result from pipetting.*

B. Materials Required, But Not Provided

1. FACS® Brand Lysing Solution 10X Concentrate from Becton Dickinson (Order #92-0002) or equivalent.
2. Phosphate Buffered Saline (PBS) from Irvine Scientific (Catalog #9242) or equivalent.
3. Fixing solution: PBS with 0.5% formaldehyde; add 1.35 ml 35% formaldehyde to 100 ml PBS.

C. Step-by-Step Procedure.

See DIRECTIONS FOR USE on page 2.

DIRECTIONS FOR USE

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 whole blood sample into a 12 x 75 mm tube.
3. Pipette 2 µl anti-Class I FITC conjugate into the tube and mix well with gentle vortex. Antibody can be diluted 1:5 in PBS. Use 10 µl diluted antibody for 100 µl whole blood. Dilute only the amount needed. Discard unused portion of diluted antibody. Do not store.
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 cytometry analysis, or can be stored in the dark at 2 –5° C for up to 24 hours before being analyzed.

LIMITATIONS OF THE PROCEDURE

1. EDTA is the anticoagulant of choice. However, ACD or sodium heparin can also be used.
2. Sterile blood samples should be stored at room temperature and analyzed within 3 days of colleciton.
3. The volume of reagent recommended is based on studies of normal human blood.
4. Laboratories using procedures and/or instruments other than those indicated may need to adjust the volume of reagent needed for each sample in order to obtain best results.

EXPECTED VALUES

Normal human lymphocyte samples will have a gated mean channel of >100, 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.

DATA ANALYSIS

1. Align and quality control the flow cytometer daily, according to the manufacturer's recommended start-up procedure.
2. Collect a green fluorescence 5,000 – 10,000 events for each sample.
3. Gate around the lymphocyte region and obtain peak channel and mean.

ABBREVIATIONS USED

ACD	Acid Citrate Dextrose
EDTA	Etheylenediamenetetraacetic Acid
FITC	Fluorescein Isothiocyanate
FSC	Forward Scatter
HLA	Human Leukocyte Antigen

TRADEMARKS USED IN THIS DOCUMENT

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TROUBLESHOOTING

Problem	Cause	Solution(s)
1. Abnormal dot plot pattern.	a. FACS parameters are not set correctly.	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.	b. Check the lysing buffer and lysing conditions. If the correct lysis buffer and conditions were used, take 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 incubation temperature to 37° C. • Increase the lysing incubation time to 20 minutes.
2. Low signal from the positive samples.	a. The flow cytometer needs alignment. b. The whole blood may not be lysed well. c. The blood was not mixed well with the antibody.	a. Re-align the instrument. b. Check the dot plot of the sample. If it looks abnormal or the histogram shows two peak, lyse the sample again. Refer to **TROUBLESHOOTING**, 1b. c. Make sure the blood is mixed well with the antibody.

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

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