

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

CONJUGATED MONOCLONAL ANTIBODY HLA and Non-HLA Specific Biotin or FITC Conjugated Monoclonal Antibodies

For Research Use Only. Not for use in diagnostic procedures.

Catalog Numbers

Specificity	Isotype	Biotin Conjugated Cat. #	FITC Conjugated Cat. #	Specificity	Isotype	Biotin Conjugated Cat. #	FITC Conjugated Cat. #
A1,36	IgM	BIH0331	-----	B57,58	IgM	BIH0243	-----
A2	IgM	BIH0648	-----	Bw4	IgG2a	BIH0007	FH0007
A2,28	IgG2a	BIH0037	FH0037	Bw6	IgG3	BIH0038	FH0038
A3	IgM	BIH0269	-----	DR1,10,103	IgM	BIH0126	-----
A9	IgG2b	BIH0964	FH0964	DR2	IgM	BIH0596	-----
A9	IgM	BIH0238	-----	DR3	IgM	BIH0085	-----
A11	IgM	BIH0084	-----	DR4	IgM	BIH0453B	-----
A25,26	IgM	BIH0048	-----	DR7	IgM	BIH0108	-----
A29	IgM	BIH0155	-----	DR9,14	IgM	BIH0154	-----
A30,31	IgM	BIH0087	-----	DR 12	IgM	BIH0193	-----
B5,7801	IgM	BIH0209	-----				
B15,57	IgM	BIH0507	-----				
B7, 27	IgG	BIH1453	FH1453				
B8	IgG2b	BIH0536A	FH0536A	Class I	IgG1	BIH1112	-----
B12	IgG2b	BIH0066	FH0066	Class I	IgG2b	-----	FH1197
B13	IgM	BIH0261	-----	Class II	IgG2a	BIH0002	FH0002
B13,15	IgM	BIH0129	-----	β_2 -microglobulin	IgG1	-----	FH1078
				Granulocyte	IgM	-----	FH0061

Non-HLA Antibodies			
CD ₂₀	IgG2a	-----	FH1079
CD ₃	IgG1	-----	FH1080

INTENDED USE

These reagents are used for direct immunofluorescence staining of specific cell surface antigens with analysis by flow cytometry. Antibodies can also be used for the detection of HLA antigens on the lymphocytes in whole blood samples and cells isolated from blood or lymphoid tissue.

SUMMARY AND EXPLANATION

The conjugated murine monoclonal antibodies listed above can be used for the detection of specific cell surface antigens listed in the table above.

PRINCIPLE

Conjugated monoclonal antibodies (mAbs) detect cells bearing the cell surface antigens specific to the antibody. Cells (whole blood or isolated cells) are stained with the conjugated mAb, red blood cells are lysed, and white blood cells are fixed with formaldehyde. Flow cytometric analysis is performed on the target cells

REAGENTS

A. Identification

Monoclonal Antibody	Antibody Concentration	Total Volume	F:P Ratio
HLA Antigen Specific-FITC (catalog numbers with FH prefix)	0.1 mg/ml - 0.5 mg/ml	250 μ l (25 tests)	4.0 – 8.0
HLA Antigen Specific-Biotin (catalog numbers with BIH prefix)	0.1 – 0.5 mg/ml	250 μ l (25 tests)	N/A
CD20-FITC (OLI Cat. #FH1079)	0.1 mg/ml	250 μ l (25 tests)	4.0 – 8.0
CD3-FITC (OLI Cat. #FH1080)	0.1 mg/ml	250 μ l (25 tests)	4.0 – 8.0

B. Warning or Caution

1. For Research Use Only. Not for use in diagnostic procedures.
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 known 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 Material Safety Data Sheet for detailed information.

C. Instructions for Use

See "Directions for Use" in box below.

D. Storage Instructions

FITC and Conjugated Monoclonal Antibodies are light sensitive and should be stored in the dark. Store reagents at 2 - 5°C.

E. Purification or Treatment Required for Use

1. To avoid volume loss, centrifuge conjugated mAb vial for a few seconds in a microcentrifuge before opening (liquid may accumulate in cap during shipment).
2. Vial is susceptible to volume loss if left uncovered. **Cover vial immediately after use!**

F. Instability Indications

Caution: Do not use the reagent if precipitate is observed.

SPECIMEN COLLECTION AND PREPARATION

- A. The blood specimen should be collected in an ACD (Acid Citrate Dextrose) Vacutainer® blood collection tube and analyzed within three days. However, EDTA (K3) or Sodium Heparin may also be used.
- B. Sterile blood samples should be stored horizontally at room temperature (20 - 25°C) and analyzed within 3 days of collection.

PROCEDURE

A. Materials Provided

FITC or Biotin Conjugated Monoclonal Antibody.

B. Materials Required, But Not Provided

- Red Blood Cell lysing solution (Do not use ammonium chloride solution).
- Streptavidin-PE (Jackson Immuno Research or equivalent)
- Phosphate Buffered Saline (PBS) or equivalent
- Fixing solution: PBS with 0.5% formaldehyde (add 1.35 ml 37% formaldehyde to 100 ml PBS).

C. Step-by-step procedure.

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

DIRECTIONS FOR USE

Note: To avoid volume loss, centrifuge conjugated monoclonal antibodies vial for a few seconds in a microcentrifuge before use (liquid may accumulate in cap during shipment).

A. Whole Blood Staining Method

1. Pipette 100 µl of whole blood sample into a 12 x 75 mm tube.
2. Pipette 10 µl of conjugated mAb into the tube and mix well with gentle vortex. **(Reagent is susceptible to volume loss if vial is left uncovered. Cover vial immediately after use.)**
3. Incubate in the dark at 2 - 5°C for 30 minutes with gentle shaking.
4. Lyse Red Blood Cells.
5. Centrifuge samples at 300 g for 5 minutes. Discard the supernatant.
6. Resuspend pellet in 2 ml of PBS, mix, and centrifuge at 300 g for 5 minutes. Discard the supernatant.
7. For FITC conjugated mAb, go to step #9. For Biotin conjugated mAb, add 100 µl streptavidin-PE according to manufacturer's recommended dilution. (As an option, 10µl of CD₃-FITC (Cat #FH1079) or CD₂₀-FITC (Cat #FH1080) can be added to the streptavidin-PE for simultaneously staining T cell or B cell population.) Incubate for 30 minutes in the dark at 2 - 5°C. Add 2 ml of PBS.
8. Repeat steps 5 and 6 twice.
9. 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.

B. Isolated Cell Staining Method

1. Isolate leukocytes from heparinized blood or tissue samples by Ficoll-Hypaque density gradient centrifugation.
2. Wash the cells twice with McCoy's and centrifuge at 300 g for 5 minutes.
3. Resuspend the cells in McCoy's and adjust the concentration to 1×10^6 cell/ml.
4. Incubate 100 μ l (10^5 cells) with 10 μ l of conjugated mAb in the dark at 2 - 5°C for 20 minutes.
5. Wash the cells twice with 2 ml PBS at 300 g for 5 minutes.
6. For FITC conjugated mAb, go to step #8. For Biotin conjugated mAb, add 100 μ l streptavidin-PE according to manufacturer's recommended dilution. Incubate for 30 minutes in the dark at 2 - 5°C.
7. Repeat step 5.
8. 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 24 hours before being analyzed.

RESULTS

See EXPECTED VALUES below.

LIMITATIONS OF THE PROCEDURE

1. Freezing isolated cells may decrease the signal.
2. The volume of HLA-Class I FITC-conjugated mAb reagent recommended is based on studies of normal human blood.

EXPECTED VALUES

Each laboratory should establish the normal ranges of positive cells and negative cells under its own testing conditions for each batch of samples. FITC conjugated monoclonal antibodies should show a FL1 channel shift where it reacts with the positive cells when compared to the negative cells.

Biotin conjugated monoclonal antibodies combined with the usage of streptavidin-PE should show a FL2 channel shift when reacting with the positive cells.

Different FITC or Biotin conjugated monoclonal antibodies may have different ranges of channel shift for the positive reactions. In Addition, individual lots of FITC or Biotin conjugated monoclonal antibodies may have slightly different channel shifts for the positive reactions.

SPECIFIC PERFORMANCE CHARACTERISTICS

Normal lymphocytes should all express HLA-Class I antigens. Therefore, lymphocytes should be selected by proper gates on FSC versus SSC dot plots for the studies of HLA-Class I antigens.

B cells express both Class I and Class II antigen, whereas T cells do not express class II antigens. Therefore, when Class II antigens are studied using the biotin conjugated monoclonal antibody, selection of B cells using CD20-FITC with streptavidin-PE is the preferred method.

BIBLIOGRAPHY

1. NCCLS Tentative Standard. "Leukocyte Differential Counting." Publication Number H20-T, NCCLS, Vol. 4, No. 11 (1984).
2. R Pei et al. A monospecific HLA-27 fluorescein isothiocyanate conjugated monoclonal antibody for rapid, simple, and accurate HLA-B27 typing. Tissue Antigen 1993; 41:200-203.
3. R. Pei, G. Woo and J.H. Lee. Detection of Blood Chimerism at a Frequency of One Per Thousand by Flow Cytometry. Visuals of the Clinical Histocompatibility Workshop, 1995, Paul I.Terasaki, Ed., pp. 73-74.
4. R. Pei, T. Chen, J. Orpilla, and J.H. Lee. A simultaneous negative and positive selection method that can detect chimerism at a frequency of 1 per 10,000 by flow cytometry. Tissue Antigens 1997; 50: 197-201.