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ProductInformation

**MONOCLONAL ANTI-HUMAN HLA CLASS I ANTIGEN
CLONE W6/32
FITC Conjugate
Purified Mouse Immunoglobulin**

Product No. **F 5662**

Product Description

Monoclonal Anti-Human HLA Class I Antigen (mouse IgG2a isotype) is derived from the hybridoma produced by the fusion of mouse myeloma cell line NS-1 and splenocytes from BALB/c mice immunized with human tonsil leukocyte membrane preparation. The isotype is determined using Sigma Immunosort™ Kit (Product Code ISO-1) and by a double diffusion assay using Mouse Monoclonal Antibody Isotyping Reagents (Product Code ISO-2). The product is prepared by conjugation of fluorescein isothiocyanate (FITC) Isomer I to purified HLA Class I Antigen monoclonal antibody. The conjugate is then purified by gel filtration to remove unbound FITC, no free FITC is detectable.

FITC Monoclonal Anti-Human HLA Class I Antigen may be used for:

1. Immunochemical analysis of HLA Class I antigens.
2. Studies of HLA-A,B,C expression on embryonic and neoplastic tissues.
3. Purification of HLA-A,B,C antigens.

FITC Monoclonal Anti-Human HLA Class I Antigen^{1,2} recognizes the human HLA class I antigen expressed on most human nucleated cells. The product specifically reacts with a monomorphic epitope on the 44-45 kD α -chain polypeptide of human class I HLA molecules (HLA-A,B,C). This glycosylated polypeptide is non-covalently associated with the 12 kD invariant β_2 -microglobulin to form the heterodimer HLA-A,B,C class I antigen of the human major histocompatibility complex (MHC). The two N-terminal domains of each α chain of class I molecules are folded in such a way that two α -helical structures on top of a base plate of β pleated sheets form a groove into which fit degraded molecules such as peptides.^{1,2,3} HLA class I antigens are widely distributed on human nucleated cells.^{1,2,3,4} Expression intensity varies considerably on different cell types. Thyroid and muscle cells are only weakly positive, while exocrine, pancreas and intestinal villous trophoblastic cells are negative. Neuronal cells in the absence of activation by cytokines are also class I negative.

The function of HLA class I molecules is to present antigen to CD8⁺ lymphocytes. They are involved in the regulation of positive and negative selection of CD8⁺ T cells by thymic epithelial cells during T cell maturation. These molecules are important in allograft rejection and in the linkage of certain haplotypes in autoimmune diseases. Expression of HLA class I antigens may be modulated in pathological states. Malignant cells may lose these antigens while hepatocytes in alcoholic hepatitis, biliary cirrhosis and chronic active hepatitis may display enhanced reactivity. Increased expression of these antigens is described in muscle cells affected by muscular dystrophy, inflammatory myopathy and other neuro-muscular disorders. The antibody may be used in immunoprecipitation, complement mediated cytotoxicity, flow cytometry and immunohistochemical staining of acetone-fixed, frozen sections and cell smears. It cross-reacts with cells from African and Asian apes and old world monkeys. No reactivity is seen with cells from most new world monkeys or from non-primates. The epitope recognized by this antibody is sensitive to routine formalin-fixation and paraffin-embedding.

Reagents

The conjugate is provided in 0.01 M phosphate buffered saline, pH 7.4, containing 1% BSA with 15mM sodium azide as a preservative.

Precautions and Disclaimers

Due to the sodium azide content, a material safety data sheet (MSDS) for this product has been sent to the attention of the safety officer of your institution. Consult the MSDS for information regarding hazardous and safe handling practices.

Product Profile

When assayed by flow cytometric analysis, using 10 μ l of the antibody to stain 1×10^6 cells, a fluorescent intensity is observed similar to that obtained with saturating monoclonal antibody levels. The percent population positive is also at the maximum percentage positive using saturating monoclonal antibody levels.

A minimum working dilution of 1:10 is determined by direct immunofluorescent staining using acetone-fixed, frozen sections of human tonsil.

F/P Molar Ratio: 3 to 8

In order to obtain best results in different preparations, it is recommended that each individual user determine their optimum working dilutions by titration assay.

Storage

Store at 2-8 °C. Protect from prolonged exposure to light. If slight turbidity occurs upon prolonged storage, clarify the solution by centrifugation before use.

Procedure for Direct Immunofluorescent Staining

Reagents and Materials Needed but Not Supplied

1. a. Whole human blood collected by standard clinical blood evacuation tubes with EDTA, ACD-A or heparin anticoagulant **or**
b. Human cell suspension (e.g., peripheral blood mononuclear cells isolated on HISTOPAQUE® (Product Code. 1077-1)).
2. Diluent: 0.01 M phosphate buffered saline (PBS), pH 7.4, containing 1% BSA and 0.1% NaN₃.
3. FITC conjugated, isotype-matched, non-specific mouse immunoglobulin (negative control, Product No. F 6522).
4. 12 x 75 mm test tubes.
5. Adjustable micropipet.
6. Centrifuge.
7. Counting chamber.
8. Trypan blue (Product No. T 0776), 0.2% in 0.01 M PBS, pH 7.4.
9. 2% paraformaldehyde in PBS.
10. Whole blood lysing solution.
11. Flow cytometer.

Procedure

1. a. Use 100 µl of whole blood **or**
b. Adjust cell suspension to 1×10^7 cells/ml in diluent. Cells should be >90% viable as determined by dye exclusion (e.g., trypan blue). For each sample, add 100 µl or 1×10^6 cells per tube.

2. Add 10 µl of conjugate to tube(s) containing cells to be stained. Vortex tube gently. Incubate the cells at room temperature (18 – 22 °C) for 30 minutes. Proper controls to be included for each sample are:
 - a. An autofluorescence control: 10 µl diluent in place of monoclonal antibody, followed by steps 3 - 7.
 - b. A negative staining control: 10 µl of FITC conjugated, isotype-matched non-specific mouse immunoglobulin (Product No. F6522) at the same concentration as test antibody followed by steps 3 - 7.
3. a. If whole blood is used, use lysing solution after incubation and wash cells according to manufacturer's instructions.
b. If a mononuclear cell suspension is used, proceed to Step. 4.
4. Add 2 ml of diluent to all tubes.
5. Pellet cells by centrifugation at 500 x G for 10 minutes.
6. Remove supernatant by careful aspiration.
7. Resuspend cells in 0.5 ml of 2% paraformaldehyde. Analyze in a flow cytometer according to manufacturer's instructions.

Quality Control

It is advisable to run the appropriate negative controls. Negative controls establish background fluorescence and non-specific binding of the primary and secondary antibodies. The ideal negative control reagent is a mouse monoclonal or myeloma protein that has no reactivity with human cells. It should be isotype-matched to the antibody and of the same concentration and F/P molar ratio as the antibody. The degree of autofluorescence or negative control reagent fluorescence will vary with the type of cells under study and the sensitivity of the instrument used.

References

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2. Brodsky, F., et al., Immunol. Rev., **47**, 3 (1979).
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4. Daar, A., et al., Transplantation, **38**, 287 (1984).

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