



Product Information

MONOCLONAL ANTI-HUMAN HLA CLASS I ANTIGEN Clone W6/32 Purified Mouse Immunoglobulin

Product No. H 1650

Product Description

Monoclonal Anti-Human HLA Class I Antigen (mouse IgG2a isotype) is derived from the W6/32 hybridoma produced by the fusion of mouse myeloma cells and splenocytes from BALB/c mice immunized with human HLA class I antigen. The isotype is determined using Sigma ImmunoType™ Kit (Product Code. ISO-1) and by a double diffusion immunoassay using Mouse Monoclonal Antibody Isotyping Reagents (Productock No. ISO-2).

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. Thus thyroid and muscle cells are only weakly positive, while exocrine pancreas and 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.

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.

Reagents

The product is provided as purified antibody (200 μ g/ml) in 0.01 M phosphate buffered saline, pH 7.4, containing 1% BSA and 15 mM sodium azide as a preservative.

Precautions/Disclaimer

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.

Performance

When assayed by flow cytometric analysis, using 5 μ l of the antibody to stain 1×10^6 cells, a fluorescence 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 working dilution of 1:10 was determined by indirect immunofluorescent staining using acetone-fixed, frozen sections of human tonsil.

Storage/Stability

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

Procedure for Indirect Immunofluorescent Staining Reagents and Materials Needed but Not Supplied

- Whole human blood collected by standard clinical blood evacuation tubes with EDTA, ACD-A or heparin anticoagulant **or**
 - Human cell suspension (e.g. peripheral blood mononuclear cells isolated on HISTOPAQUE® (Sigma Stock No. 1077-1)).
- Diluent: 0.01 M phosphate buffered saline (PBS), pH 7.4, containing 1% BSA and 0.1%NaN₃.
- Fluorochrome (FITC, PE, or Quantum Red™) conjugated anti-mouse secondary antibody diluted to recommended working dilution in diluent (e.g. Product No. F2883 FITC-Sheep Anti-Mouse IgG (whole molecule), F(ab')₂ fragment of Affinity Isolated Antibody). Aggregates in conjugates should be removed by centrifugation immediately prior to use.
- Isotype-matched, non-specific mouse immunoglobulin (negative control, Product No. M5409).
- 12 x 75 mm test tubes.
- Adjustable micropipet.
- Centrifuge.
- Counting chamber.
- 0.2% Trypan blue (Product No. T0776) in 0.01 M phosphate buffered saline, pH 7.4.
- 2% paraformaldehyde in PBS.
- Whole blood lysing solution.
- Flow cytometer.

Procedure

- Use 100 µl of whole blood **or**
 - Adjust cell suspension to 1 x 10⁷ 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 x 10⁶ cells per tube.
- Add 5 µl of monoclonal antibody 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:

- Autofluorescence control: 5 µl diluent in place of monoclonal antibody.
- Negative staining control 1:5 µl isotype-matched non-specific mouse immunoglobulin (Product No. M5409) at the same concentration as test antibody.

- After 30 minutes, add 2 ml of diluent to all tubes.
- Pellet cells by centrifugation at 500 x G, for 10 minutes.
- Remove supernatant by careful aspiration.
- Resuspend the cells in 100 µl of the fluorochrome conjugated secondary antibody at the recommended concentration. For the autofluorescence control, add 100 µl of diluent. Incubate at room temperature (18 - 22 °C) for 30 minutes. Protect from light at this and all subsequent steps.
- If whole blood is used, use lysing solution according to manufacturer's instructions after incubation.
 - If a mononuclear cell suspension is used, proceed to Step. 8.
- Add 2 ml diluent to all tubes.
- Wash as in steps 4-5.
- After last wash, resuspend cells in 0.5 ml of 2% paraformaldehyde and 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 staining of the primary and secondary antibodies. The ideal negative control reagent is a mouse monoclonal or myeloma protein. It should be isotype-matched to the primary antibody, not specific for human cells and of the same concentration as the primary 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.

For fluorescence analysis of cells with Fc receptors, the use of isotype matched negative control is mandatory. In some systems it may be necessary to incubate the cells in 10-20% normal serum from the second antibody host species (at step 2 before adding monoclonal antibody) in order to decrease non-specific staining with the conjugated second antibody.

References

1. Barnstable, C., et al., Cell, **14**, 9 (1978).
2. Brodsky, F., et al., Immunol. Rev., **47**, 3 (1979).
3. Lawlor, D., et al., Ann. Rev. Immunol., 8, **23** (1990).
4. Daar, A., et al., Transplantation, **38**, 287 (1984).

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