Monoclonal Anti-human HLA Class I-PerCP

Catalog Number: FAB7098C
Lot Number: ACND01
100 Tests

Reagents Provided

Peridinin-Chlorophyll-Protein-Complex (PerCP)-conjugated mouse monoclonal anti-human HLA Class I: Supplied as 25 µg of antibody in 1 mL saline containing up to 0.5% BSA and 0.1% sodium azide.

Clone #: W6/32

Isotype: mouse IgG_{2A}

Reagents Not Provided

- Flow Cytometry Staining Buffer (Catalog # FC001) or other BSA-supplemented saline buffer.

Storage

Reagents are stable for **twelve months** from the date of receipt when stored in the dark at 2° - 8° C.

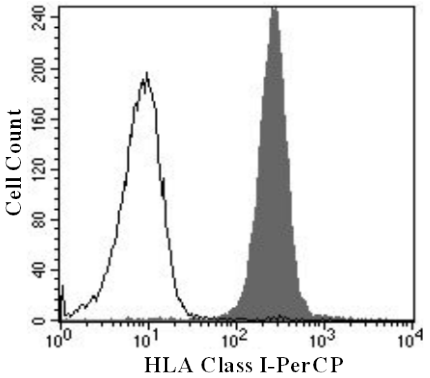
Intended Use

Designed to quantitatively determine the percentage of cells bearing HLA Class I within a population and qualitatively determine the density of HLA Class I on cell surfaces by flow cytometry.

Product Description

This antibody detects the human major histocompatibility complex (MHC) class I, HLA-A, B, and C. It recognizes a non-polymorphic epitope shared among products of the HLA-A, B, and C loci and immunoprecipitates both the HLA molecule and beta 2-Microglobulin.

The IgG fraction of a hybridoma tissue culture supernatant was purified by Protein A or G affinity chromatography. The purified antibody was then conjugated to PerCP fluorochrome. Cell surface expression of HLA Class I is determined by flow cytometry. PerCP has a maximum absorption of 482 nm and 564 nm and a maximum emission of 675 nm.



Human whole blood granulocytes were stained with PerCP-conjugated anti-human HLA Class I (Catalog # FAB7098C, filled histogram) or PerCP-conjugated isotype control (Catalog # IC003C, open histogram).

Background Information

HLA-A, B, and C are approximately 45 kDa transmembrane glycoproteins in the major histocompatibility complex I (MHC I) family. They contain three alpha domains in their extracellular regions. HLA molecules are expressed on nearly all nucleated cells in association with the 12 kDa beta 2-Microglobulin. This complex binds peptides derived from pathogenic cytosolic or extracellular proteins such as viral or microbial proteins. It presents these peptides on the cell surface for recognition by the T cell receptor on $CD8^+$ cytotoxic T cells. The activated cytotoxic T cell then kills the presenting cell. Mismatched MHC I alleles between a host and a donor lead to transplant rejection.

Reference

1. Barnstable, C.J. *et al.* (1978) Cell **14**:9.

Flow Cytometry Validation

This antibody has been tested for flow cytometry using human whole blood granulocytes.

1. Cells may be Fc-blocked with 1 µg of human IgG/10^5 cells for 15 minutes at room temperature. Do not wash excess blocking IgG from this reaction.
2. After blocking, 10 µL of conjugated antibody was added to 1 - 2.5 x 10^5 cells and incubated for 30 minutes at room temperature.
3. Unbound antibody was removed by washing the cells twice in Flow Cytometry Staining Buffer (Catalog # FC001). Note that whole blood requires a RBC lysis step at this point using Flow Cytometry Human Lyse Buffer (Catalog # FC002).
4. The cells were resuspended in Flow Cytometry Staining Buffer for final flow cytometric analysis. As a control for this analysis, cells in a separate tube should be treated with PerCP-labeled mouse IgG_{2A} antibody. This procedure may need to be modified, depending upon the cell type and final utilization. Individual users may need to titrate to determine the optimal reagent amount for their specific use.

Warning: Contains sodium azide as a preservative - sodium azide may react with lead and copper plumbing to form explosive metal azides. Flush with large volumes of water during disposal.

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