Monoclonal Anti-Integrin-α6/CD49f-APC

Catalog Number: FAB13501A
Lot Number: LMP04
100 Tests

Reagents Provided

Allophycocyanin (APC)-conjugated rat monoclonal anti-human/bovine/mouse Integrin-α6/CD49f: Supplied as 10 μg of antibody in 1 mL saline containing up to 0.5% BSA and 0.1% sodium azide.

Clone #: GoH3

Isotype: rat IgG_{2A}

Reagents Not Provided

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

Storage

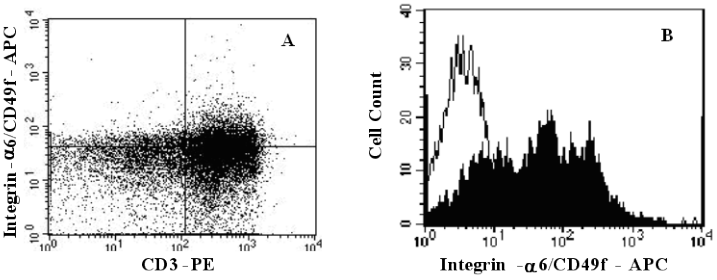
Reagents are stable for **twelve months** from the date of receipt when stored in the dark at 2-8 °C.

Intended Use

Designed to quantitatively determine the percentage of cells bearing Integrin-α6/CD49f within a population and qualitatively determine the density of Integrin-α6/CD49f on cell surfaces by flow cytometry.

Product Description

This antibody was produced from a hybridoma resulting from the fusion of a mouse myeloma with B cells obtained from a rat immunized with mouse mammary tumor cells. The IgG fraction of the tissue culture supernatant was purified by Protein G affinity chromatography. The purified antibody was then conjugated to APC fluorochrome. Cell surface expression of Integrin-α6/CD49f is determined by flow cytometry using 620-650 nm wavelength excitation and monitoring emitted fluorescence with a detector optimized to collect peak emissions at 660-670 nm.



A) Mouse splenocytes were stained with APC-conjugated anti-human/bovine/mouse Integrin-α6/CD49f (Catalog # FAB13501A) and PE-conjugated anti-mouse CD3 (Catalog # FAB4841P). Quadrant markers were set based on staining with an APC-conjugated isotype control (Catalog # IC006A). **B)** Human whole blood lymphocytes were stained with APC-conjugated anti-human/bovine/mouse Integrin-α6/CD49f (filled histogram) or isotype control (open histogram).

Background Information

Integrin-α6 is a membrane associated protein of 1050 amino acids that normally forms heterodimers with a variety of cell surface proteins.[1, 2] Integrin-α6, also known as CD49f, associates with the Integrin-β1 chain (CD29) to form VLA-6 and with the Integrin-β4 chain (CD104) to form the Integrin-α6β4 complex, also known as the laminin and kalinin receptor.[3] It is expressed mainly on T cells, monocytes, platelets, epithelial and endothelial cells, perineural cells, and trophoblasts of placenta.[4-6] Additional studies have also shown Integrin-α6 expression on germinal center B cells.[7] There is a high degree of amino acid homology (93%) between the human and mouse Integrin-α6 molecules and this explains why this monoclonal antibody stains cells from different species.[8, 9]

References:

1. Tamura, R.N. *et al.* (1990) J. Cell. Biol. **111**:1593.
2. Hogervost, F. *et al.* (1991) Eur. J. Biochem. **199**:425.
3. Knapp, W.B. *et al.* (1989) Leucocyte Typing IV: White Cell Differentiation Antigens, Oxford University Press, New York.
4. Botling, J. *et al.* (1995) Leukemia **9**:2034.
5. Wu, J.E. & S.A. Santoro (1996) Dev. Dyn. **206**:169.
6. Kaur, P. & A. Li (2000) J. Invest. Dermatol. **114**:413.
7. Ambrose, H.E. & S.D. Wagner (2004) Immunology **111**:400.
8. Sonnenberg, A. *et al.* (1987) J. Biol. Chem. **262**:10376.
9. Uematsu, J. *et al.* (1994) J. Biochem. (Tokyo) **115**:469.

Flow Cytometry Validation

This antibody has been tested for flow cytometry using mouse splenocytes and human whole blood lymphocytes.

1. Cells may be Fc-blocked with 1 μg of mouse 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 up to 1 x 10^6 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 Mouse Lyse Buffer (Catalog # FC003).
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 APC-labeled rat 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.