Monoclonal Anti-human/mouse CD109-Fluorescein

Catalog Number: FAB4385F
Lot Number: ABND01
100 Tests

Reagents Provided

Carboxyfluorescein (CFS)-conjugated mouse monoclonal anti-human/mouse CD109: Supplied as 25 µg of antibody in 1 mL saline containing up to 0.5% BSA and 0.1% sodium azide.

Clone #: 496920

Isotype: mouse IgG_{2A}

Reagents Not Provided

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

Storage

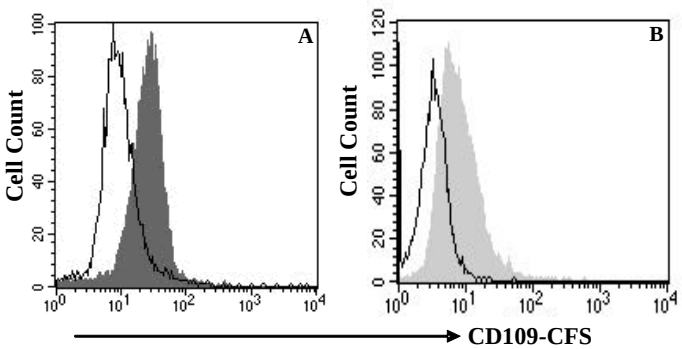
Reagents are stable for **twelve months** from the date of receipt when stored in the dark at 2° - 8° C.

Intended Use

Designed to quantitatively determine the percentage of cells bearing CD109 within a population and qualitatively determine the density of CD109 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 mouse immunized with purified, NS0-derived, recombinant human CD109 (rhCD109; aa 22 - 1268; Accession # Q6YHK3). The IgG fraction of the tissue culture supernatant was purified by Protein G affinity chromatography. The purified antibody was then conjugated to CFS fluorochrome. Cell surface expression of CD109 is determined by flow cytometry using 488 nm wavelength excitation and monitoring emitted fluorescence with a detector optimized to collect peak emissions at 515 - 545 nm.



The A431 human cell line (A) and PMA/ionomycin-stimulated mouse splenocytes (B; gated on $CD3^+$ cells) cells were stained with CFS-conjugated anti-human/mouse CD109 (Catalog # FAB4385F, filled histogram) or CFS-conjugated mouse isotype control (Catalog # IC003F, open histogram).

Background Information

CD109 is a GPI-anchored member of the α_2-macroglobulin (A2M) and complement family of proteins.[1] Mature human CD109 contains a bait region with recognition sequences for multiple proteases, an internal thioester bond, and a domain similar to the receptor binding domain of A2M.[2] Cleavage of A2M family proteins within the bait region activates the thioester bond to promote covalent bonding to nucleophilic groups in adjacent molecules.[3, 4] Within the region included in the recombinant protein used for immunization, human CD109 shares 71% - 73% amino acid (aa) sequence identity with mouse and rat CD109. CD109 is expressed on activated T cells and platelets, hematopoietic stem cells, megakaryocyte precursors, vascular endothelial cells, basal and myoepithelial cells of secretory glands, and squamous cell carcinomas.[2 - 5]

References

1. Travis, J. & G.S. Salvesen (1983) Annu. Rev. Biochem. **52**:655.
2. Lin, M. *et al*. (2002) Blood **99**:1683.
3. Christensen, U. & L. Sottrup-Jensen(1984) Biochemistry **23**:6619.
4. Wallis, R. *et al*. (2007) J. Biol. Chem. **282**:7844.
5. Murray, L.J. *et al*. (1999) Exp. Hematol. **27**:1282.

Flow Cytometry Validation

This antibody has been tested for flow cytometry using the A431 human cell line and mouse splenocytes.

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 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 Human Lyse Buffer (Catalog # FC002).
4. The cells were resuspended in Flow Cytometry Staining Buffer for flow cytometric analysis. As a control, cells in a separate tube should be treated with CFS-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.

R&D Systems Inc.
1-800-343-7475