Monoclonal Anti-mouse 4-1BB/TNFRSF9-Phycoerythrin

Catalog Number: FAB937P
Lot Number: ABSJ01
100 Tests

Reagents Provided

Phycoerythrin (PE)-conjugated rat monoclonal anti-mouse 4-1BB/TNFRSF9: Supplied as 25 μg of antibody in 1 mL saline containing up to 0.5% BSA and 0.1% sodium azide.

Clone #: 158332

Isotype: rat IgG_{2A}

Reagents Not Provided

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

Storage

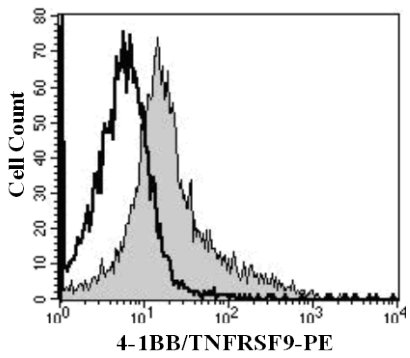
Reagents are stable for **twelve months** from the date of receipt when stored in the dark at 2° - 8° C.

Intended Use

Designed to quantitatively determine the percentage of cells bearing 4-1BB/TNFRSF9 within a population and qualitatively determine the density of 4-1BB/TNFRSF9 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 mouse 4-1BB/TNFRSF9 extracellular domain (Accession # P20334). The IgG fraction of the tissue culture supernatant was purified by Protein A or G affinity chromatography. The purified antibody was then conjugated to PE fluorochrome. Cell surface expression of 4-1BB/TNFRSF9 is determined by flow cytometry using 488 nm wavelength excitation and monitoring emitted fluorescence with a detector optimized to collect peak emissions at 565 - 605 nm.



Mouse splenocytes activated for 3 days with Concavalin A were stained with PE-conjugated anti-mouse 4-1BB/TNFRSF9 (Catalog # FAB937P, filled histogram) or PE-conjugated isotype control (Catalog # IC006P, open histogram).

Background Information

4-1BB, also known as CD137 and ILA (induced by lymphocyte activation), is a TNF receptor superfamily member and has been designated TNFRSF9. Mouse 4-1BB cDNA encodes a 256 amino acid (aa) residue type I transmembrane protein.[1-3] A soluble 4-1BB is released from the surfaces of cells expressing the transmembrane protein.[4] Mouse 4-1BB shares approximately 60% aa sequence identity with its human counterpart. 4-1BB is expressed on activated $CD4^+$ and $CD8^+$ T cells, thymocytes, and NK cells. It is also expressed on monocytes, neutrophils, dendritic cells (DCs), and eosinophils.[5] The ligand for 4-1BB (4-1BBL), also named TNFSF9, belongs to the TNF ligand superfamily. *In vivo,* the co-stimulatory activity of 4-1BB has been shown to be important in graft-vs-host disease and antiviral CTL responses. On dendritic cells, 4-1BB is a DC-activating molecule that enhances cytokine production and up-regulates expression of B7-1 and B7-2 co-stimulatory molecules, resulting in an improved ability to stimulate T cell responses.[1-5]

References

1. Goodwin, R.G. *et al*. (1993) Eur. J. Immunol. **23**:2631.
2. Alderson, M.R. *et al*. (1994) Eur. J. Immunol. **24**:2219.
3. Kwon, B.S. & S.M. Weissman (1989) Proc. Nat. Acad. Sci. USA **86**:1963.
4. Wilcox, R.A. *et al*. (2002) J. Immunol. **168**:4262.
5. Kwon, B. *et al.* (2002) Trends Immunol. **23**:378.

Flow Cytometry Validation

This antibody has been tested for flow cytometry using mouse splenocytes.

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 PE-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.

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