Monoclonal
Anti-human CD40 Ligand/TNFSF5-PerCP

Catalog Number: FAB617C
Lot Number: ABHB01
100 Tests

Reagents Provided

Peridinin-Chlorophyll-Protein-Complex (PerCP)-conjugated mouse monoclonal anti-human CD40 Ligand/TNFSF5: Supplied as 25 μg of antibody in 1 mL saline containing up to 0.5% BSA and 0.1% sodium azide.

Clone #: 40804

Isotype: mouse IgG_{2B}

Reagents Not Provided

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

Storage

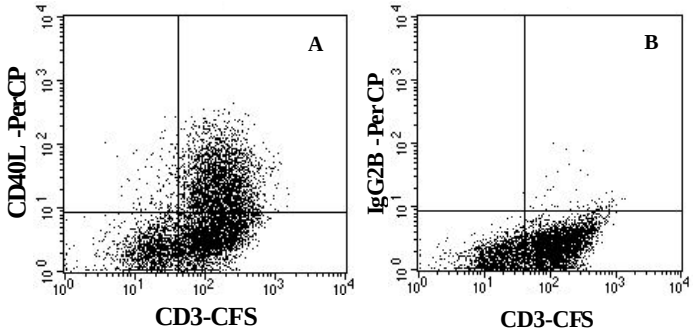
Reagents are stable for **twelve months** from the date of receipt when stored in the dark at 2° - 8° C.

Intended Use

Designed to quantitatively determine the percentage of cells bearing CD40 Ligand/TNFSF5 within a population and qualitatively determine the density of CD40 Ligand/TNFSF5 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, *E. coli*-derived, recombinant human CD40 Ligand extracellular domain (rhCD40 Ligand; aa 50 - 261; Accession # P29965). The IgG fraction of the tissue culture supernatant was purified by Protein G affinity chromatography. The purified antibody was then conjugated to PerCP fluorochrome. Cell surface expression of CD40 Ligand/TNFSF5 is determined by flow cytometric analysis. PerCP has a maximum absorption of 482 nm and 564 nm and a maximum emission of 675 nm.



Activated lymphocytes in PBMC were stained with CFS-conjugated CD3 and either A) PerCP-conjugated anti-human CD40 Ligand/TNFSF5 (Catalog # FAB617C) or B) isotype control (Catalog # IC0041C).

Background Information

CD40 Ligand is a type II transmembrane glycoprotein belonging to the TNF family (TNFSF5). It is expressed predominantly on activated $CD4^+$ T lymphocytes and also on other hematopoietic cells. CD40 L binds to CD40 (TNFRSF5) on B lymphocytes, monocytes, dendritic cells, and thymic epithelium. Engagement of CD40 by CD40 Ligand promotes B cell and dendritic cell maturation and activation.

Flow Cytometry Validation

This antibody has been tested for flow cytometry using activated lymphocytes in PBMC.

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_{2B} 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