Monoclonal Anti-human CD40 (TNFRSF5)-Phycoerythrin

Catalog Number: FAB6321P
Lot Number: LRP02
100 Tests

Reagent Information

Phycoerythrin (PE)-conjugated mouse monoclonal anti-human CD40: Supplied as 25 µg of antibody in 1 mL PBS containing 0.1% sodium azide.

Clone #: 82111

Isotype: mouse IgG_{2B}

Additional Reagents Required

- PBS (Dulbecco's PBS)
- BSA

Storage

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

Intended Use

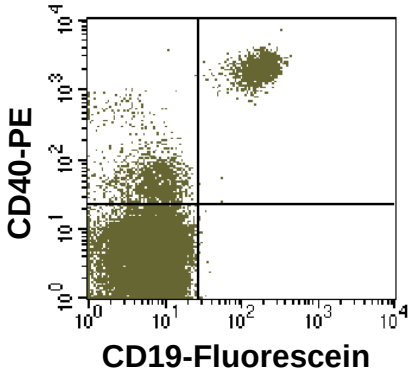
Designed to quantitatively determine the percentage of cells bearing CD40 within a population and qualitatively determine the density of CD40 on cell surfaces by flow cytometry.

Principle of the Test

Washed cells are incubated with the PE-labeled monoclonal antibody, which binds to cells expressing CD40. Unbound PE-conjugated antibody is then washed from the cells. Cells expressing CD40 are fluorescently stained, with the intensity of staining directly proportional to the density of expression of CD40. Cell surface expression of CD40 is determined by flow cytometric analysis using 488 nm wavelength laser excitation.

Reagent Preparation

PE-conjugated mouse anti-human CD40: Use as is; no preparation necessary.



Human peripheral blood lymphocytes stained with PE-conjugated anti-human CD40 antibody (Catalog # FAB6321P) and anti-human CD19-Fluorescein conjugated antibody).

Sample Preparation

Peripheral blood cells: Whole blood should be collected in evacuated tubes containing EDTA or heparin as the anticoagulant. Contaminating serum components should be removed by washing the cells three times in an isotonic phosphate buffer (supplemented with 0.5% BSA) by centrifugation at 500 x g for 5 minutes. Transfer 50 µL of packed cells to a 5 mL tube for staining with the monoclonal antibody. Whole blood will require lysis of RBC following the staining procedure.

Cell Cultures: Continuous cell lines or activated cell cultures should be centrifuged at 500 x g for 5 minutes and washed three times in an isotonic PBS buffer (supplemented with 0.5% BSA), as described above, to remove any residual growth factors that may be present in the culture medium. Cells should then be resuspended in the same buffer to a final concentration of 4 x 10^6 cells/mL and 25 µL of cells (1 x 10^5) transferred to a 5 mL tube for staining.

Note: Adherent cell lines may require pretreatment with 0.5 mM EDTA to facilitate removal from substrate. Cells that require trypsinization to enable removal from substrate should be further incubated in medium for 6 - 10 hours on a rocker platform to enable regeneration of the receptors. The use of the rocker platform will prevent reattachment to the substrate.

Sample Staining

1) Cells should be Fc-blocked by treatment with 1 µg of human IgG/10^5 cells for 15 minutes at room temperature prior to staining. Do not wash excess blocking IgG from this reaction.
2) Transfer 25 µL of the Fc-blocked cells (1 x 10^5 cells) or 50 µL of packed whole blood to a 5 mL tube.
3) Add 10 µL of PE-conjugated anti-CD40 reagent.
4) Incubate for 30 - 45 minutes at 2° - 8° C.
5) Following this incubation, remove unreacted anti-CD40 reagent by washing the cells twice in 4 mL of the same PBS buffer (*note: whole blood will require an RBC lysis step at this point using any commercially available lysing reagent, such as R&D Systems Whole Blood Lysing Kit, Catalog # WL1000*).
6) Finally, resuspend the cells in 200 - 400 µL of PBS buffer for final flow cytometric analysis.
7) As a control for analysis, cells in a separate tube should be treated with PE-labeled mouse IgG_{2B} antibody.

This procedure may need modification, depending upon final utilization.

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

Background Information

CD40 is a type I transmembrane glycoprotein that belongs to the TNF receptor superfamily (1, 2). CD40 is expressed on B cells, dendritic cells, follicular dendritic cells, activated monocytes, macrophages, endothelial cells, vascular smooth muscle cells, and several tumor cell lines (2 - 5). Interaction of CD40 with its ligand CD40L/CD154 leads to aggregation of CD40 molecules that, in turn, results in the initiation of intracellular signaling pathways (3, 5). Early studies on CD40-CD40L interactions revealed their role in humoral (6) and cell-mediated immunity (6 - 8). Interaction between CD40L on T cells and CD40 on B cells stimulates B cell proliferation and leads to immunoglobulin isotype switching (6). Cross-linking of CD40 with antibodies or by binding to CD40L produces cell type–specific responses including; costimulation and induction of proliferation, induction of cytokine production, rescue from apoptosis, and upregulation of adhesion molecules (4, 9). CD40 signaling has been linked with the pathogenesis of chronic inflammatory diseases such as multiple sclerosis and atherosclerosis (4, 10), neurodegenerative disorders, graft-vs-host disease, and cancer (4, 11).

References

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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.