Monoclonal
Anti-mouse CD40 Ligand/TNFSF5-Phycoerythrin

Catalog Number: FAB1163P
Lot Number: LRJ01
100 Tests

Reagent Information

Phycoerythrin (PE)-conjugated rat monoclonal anti-mouse 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 #: 208109

Ig class: rat IgG_{2A}

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

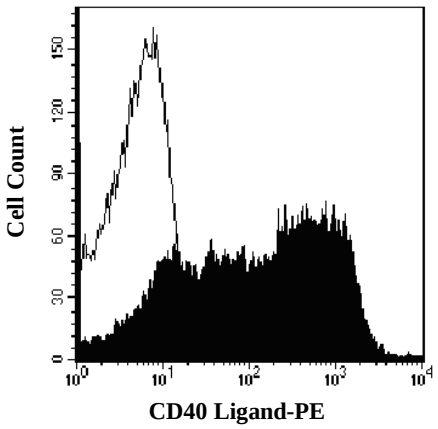
Designed to quantitatively determine the percentage of cells bearing the CD40 Ligand/TNFSF5 cell surface antigen within a population and qualitatively determine its density on cell surfaces by flow cytometry.

Principle of the Test

Washed cells are incubated with the PE-labeled monoclonal antibody that binds to the cells expressing CD40 Ligand/TNFSF5. Unbound PE-conjugated antibody is then washed from the cells. Cells expressing CD40 Ligand/TNFSF5 are fluorescently stained, with the intensity of staining directly proportional to the density of CD40 Ligand/TNFSF5. Cell surface expression of CD40 Ligand is determined by flow cytometric analysis using 488 nm wavelength laser excitation.

Reagent Preparation

Use as is; no preparation is necessary.



Activated $CD4^+$ T cells (see Technical Note) stained with PE-conjugated CD40 Ligand (Catalog # FAB1163P, filled histogram) or PE-conjugated isotype control antibody (Catalog # IC006P, open histogram).

Sample Preparation

Peripheral blood cells: Whole blood should be collected in tubes containing EDTA or heparin as the anticoagulant. Spleen cells should be first mechanically disaggregated into a single cell suspension. 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. 50 μL of packed cells are then transferred to a 5 mL tube for staining with the monoclonal. Blood cells 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 4×10^6 cells/mL and 25 μL of cells (1×10^5) are 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 to be used for staining with the antibody may be first Fc-blocked by treatment 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) Transfer 25 μL of the Fc-blocked cells (up to 1×10^6 cells) or 50 μL of packed whole blood to a 5 mL tube.
3) Add 10 μL of PE-conjugated anti-mouse CD40 Ligand reagent.
4) Incubate for 30 - 45 minutes at 2° - 8° C.
5) Following this incubation, remove unbound anti-mouse CD40 Ligand reagent by washing (described above) the cells twice in 4 mL of the same PBS buffer (*note that whole blood will require a RBC lysis step at this point using any commercially available lysing reagent, such as R&D Systems Mouse Erythrocyte Lysing Kit, Catalog # WL2000*).
6) 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 rat IgG_{2A} antibody.

This procedure may need modification, depending upon final utilization.

FOR RESEARCH USE ONLY. NOT FOR USE IN HUMANS.

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Background Information

CD40 Ligand (CD40L), also known as CD154, TRAP, or gp39, is a member of the TNF superfamily of proteins (TNFSF5). Mouse CD40L/CD154 is a 260 amino acid, single chain type-II glycoprotein (1, 2). It is expressed predominantly on activated $CD4^+$ T cells, but is also found on NK cells, mast cells, basophils and eosinophils (3). Cytokine stimulation (*e.g*. IL-1β, TNF-α, or IFN-γ) of some cell types can lead to enhanced or *de novo* expression of CD40L (4). In addition to the membrane form of CD40L, soluble forms that are monomeric, dimeric and trimeric are secreted by activated $CD4^+$ T cells (5). Both the membrane and soluble forms elicit the same biologic effects on B cells upon binding its receptor CD40 (5). However, the trimeric form of soluble CD40 has more potent biologic effects due to oligomerization of cell surface CD40, a common feature of TNF receptor family members (2, 5). CD40/CD40L interactions stimulate B cells to proliferate and leads to immunoglobulin isotype switching (6). A genetic defect in CD40L/CD154 that prevents CD40L from interacting with CD40 leads to hyper-IgM syndrome (7, 8). CD40/CD40L interactions also play a central role in the regulation of cell-mediated immunity (6, 9, 10). Cross-linking of CD40 on monocytes, macrophages, and dendritic cells induces activation, maturation, and cytokine production (11, 12). A role for CD40L has been proposed in the pathogenesis of HIV (13), systemic lupus erythematosus (14), chronic lymphocytic leukemia (15), and artherosclerosis (16).

References

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Technical Note

Purified $CD4^+$ T cells were stimulated with plate bound anti-mouse CD3 monoclonal antibody (R&D Systems, Catalog # MAB484) for 8 hours prior to staining with the PE–conjugated monoclonal antibody to CD40L/CD154.

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.