

Monoclonal Anti-human CD40 Ligand/TNFSF5 (CD154) Allophycocyanin

Catalog Number: FAB617A

Lot Number: LYJ03

100 Tests

Reagents Provided

Allophycocyanin (APC)-conjugated mouse monoclonal anti-human CD40 Ligand: Supplied as 10 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

- 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

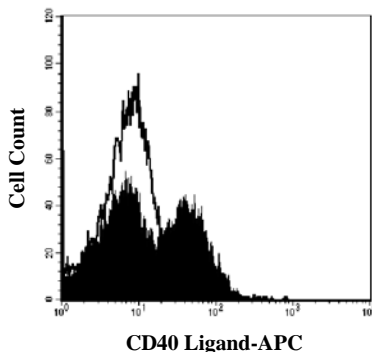
Designed to quantitatively determine the percentage of cells bearing CD40 Ligand within a population and qualitatively determine the density of CD40 Ligand on cell surfaces by flow cytometry.

Principle of the Test

Washed cells are incubated with the allophycocyanin-labeled monoclonal antibody, which binds to cells expressing CD40 Ligand. Unbound allophycocyanin-conjugated antibody is then washed from the cells. Cells expressing CD40 Ligand are fluorescently stained, with the intensity of staining directly proportional to the density of expression of CD40 Ligand. Cell surface expression of CD40 Ligand is determined by flow cytometric analysis using 620 - 650 nm wavelength laser excitation and monitoring emitted fluorescence with a detector optimized to collect peak emissions at 660 - 670 nm.

Reagent Preparation

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



Human PBMC stimulated with PMA and Ionomycin were stained with APC-conjugated anti-human CD40 Ligand (Catalog # FAB617A, filled histogram) or isotype control (Catalog # IC0041A, open histogram).

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 10⁵ cells/mL and 25 µL of cells (1 x 10⁵) 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/10⁵ 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 10⁵ cells) or 50 µL of packed whole blood to a 5 mL tube.
- 3) Add 10 µL of APC-conjugated CD40 Ligand reagent.
- 4) Incubate for 30 - 45 minutes at 2° - 8° C.
- 5) Following this incubation, remove unreacted CD40 Ligand 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 APC-labeled mouse IgG_{2B} antibody.

This procedure may need modification, depending upon final utilization.

Background Information

CD40 Ligand is a type II transmembrane glycoprotein belonging to the TNF superfamily (TNFSF5). It is expressed predominantly on activated CD4⁺ T lymphocytes and also on other hematopoietic cells. CD40 Ligand 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.

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.