Monoclonal
Anti-human CD30 Ligand/TNFSF8/CD153-Phycoerythrin

Catalog Number: FAB1028P
Lot Number: LQI03
100 Tests

Reagents Provided

Phycoerythrin (PE)-conjugated mouse monoclonal anti-human CD30 Ligand: Supplied as 25 μg of antibody in 1 mL saline containing up to 0.5% BSA and 0.1% sodium azide.

Clone #: 116614

Isotype: mouse IgG_{2B}

Reagents Not Provided

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

Storage

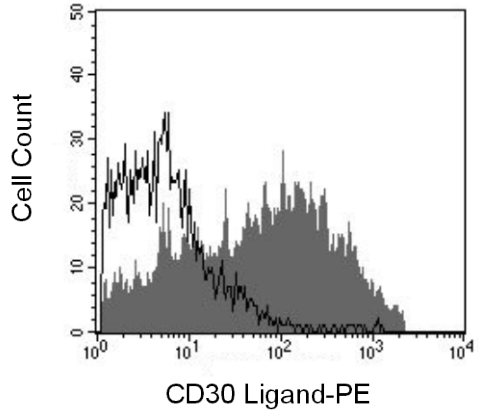
Reagents are stable for **twelve months** from date of receipt when stored in the dark at 2° - 8° C.

Intended Use

Designed to quantitatively determine the percentage of cells bearing CD30 Ligand within a population and qualitatively determine the density of CD30 Ligand 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 CD30 Ligand (rhCD30L) extracellular domain. The IgG fraction of the ascites fluid was purified by Protein G affinity chromatography. The purified antibody was then conjugated to PE fluorochrome. Cell surface expression of CD30 Ligand 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.



PMA plus Ca^{2+} ionomycin-stimulated $CD3^+$ PBMC were stained with PE-conjugated anti-human CD30L (Catalog # FAB1028P, filled histogram) or PE-conjugated isotype control (Catalog # IC0041P, open histogram).

Background Information

CD30 Ligand is a homotrimeric type II membrane protein and tumor necrosis factor superfamily (TNFSF8) member (1, 2). Also designated CD153 (1), CD30 Ligand is the specific receptor of CD30 (TNFRSF8) - a cell surface antigen of Hodgkin's and Reed-Sternberg cells (1, 3, 4). CD30 is also expressed on non-Hodgkin's lymphomas, virally infected T and B cells, and on normal T and B cells following activation (5 - 7). CD30 binding to CD30 Ligand/CD153 mediates cellular proliferation, activation, differentiation, and apoptotic cell death (2, 8, 9).

References

1. Armitage, R.J. (2000) J. Biol.Regul.Homeost.Agents.**14**:142.
2. Smith, C.A. *et al.* (1993) Cell **73**:1349.
3. Falini, B. *et al.* (1995) Blood **85**:1.
4. Schwab, U. *et al* (1982) Nature **299**:65.
5. Andreesen, R. *et al* (1984) Blood **63**:1299.
6. Stein, H. *et al* (1982) Int.J.Cancer **30**:445.
7. Stein, H. *et al* (1985) Blood **66**:848.
8. Gruss, H.J. *et al* (1996) Ann.Oncol **7**:19.
9. Cerutti, A. *et al* (2000) J. Immunol. **165**:786.

Flow Cytometry Validation

This antibody has been tested for flow cytometry using PMA plus Ca^{2+} ionomycin-activated $CD3^+$ PBMCs.

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 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 Human Lyse Buffer (Catalog # FC002).
4. The cells were resuspended in Flow Cytometry Staining Buffer for final flow cytometric analysis. As a control for analysis, cells in a separate tube should be treated with PE-labeled mouse IgG_{2B} antibody. This procedure may need to be modified, depending upon cell type and final utilization. Individual users may need to titrate to determine 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.

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