Monoclonal Anti-human ICOS-PE

Catalog Number: FAB6975P
Lot Number: ACSN01
100 Tests

Reagents Provided

Phycoerythrin (PE)-conjugated mouse monoclonal anti-human ICOS: Supplied as 25 μg of antibody in 1 mL saline containing up to 0.5% BSA and 0.09% sodium azide.

Clone #: 669222

Isotype: mouse IgG_1

Reagents Not Provided

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

Storage

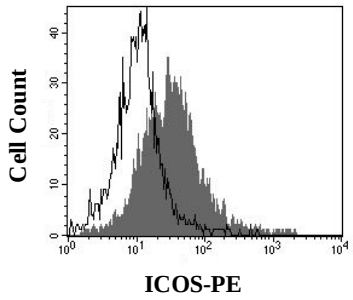
Reagents are stable for **twelve months** from the date of receipt when stored in the dark at 2-8 °C.

Intended Use

Designed to quantitatively determine the percentage of cells bearing ICOS within a population and qualitatively determine the density of ICOS 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 NS0 cells transfected with human ICOS (Accession # Q9Y6W8). The IgG fraction of the tissue culture supernatant was purified by Protein A or G affinity chromatography. The purified antibody was then conjugated to PE fluorochrome. Cell surface expression of ICOS 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.



Human $CD3^+$ PBMC were treated for 48 hours with 5 μg/mL PHA and stained with PE-conjugated anti-human ICOS (Catalog # FAB6975P; filled histogram) or PE-conjugated isotype control (Catalog # IC002P; open histogram).

Background Information

Inducible co-stimulator (ICOS), also known as AILIM (activation-inducible lymphocyte immunomediatory molecule) and CRP-1 (CD28-related protein-1), is a member of the CD28 family of immune co-stimulatory receptors. Other family members are CD28, CTLA-4, and PD-1. Human ICOS is a homodimeric type I transmembrane protein consisting of 199 amino acids (aa) with a putative 20 aa signal sequence, a 121 aa extracellular domain, a 23 aa transmembrane region, and a 35 aa cytoplasmic domain. ICOS shares approximately 39% amino acid similarity with CD28 and CTLA-4. Human and mouse ICOS share approximately 72% amino acid identity. ICOS is expressed on most $CD45RO^+$ cells. ICOS expression is up-regulated within approximately 24 - 48 hours of activation on T helper (Th) primed cells. B7-H2, a member of the B7 family of co-stimulatory ligands, has been identified as the ICOS ligand. The B7-H2/ ICOS interaction appears to play roles in T cell-dependent B cell activation and T helper cell differentiation.

References

1. Aicher, A. *et al*. (2000) J. Immunol. **164**:4689.
2. Coyle, A.J. *et al*. (2000) Immunity **13**:95.
3. Coyle, A.J. & J.C. Gutierrez-Ramos (2001) Nat. Immunol. **2**:203.
4. Gonzalo, J.A. *et al*. (2001) J. Immunol. **166**:1.
5. Hutloff, A. *et al*. (1999) Nature **397**:263.
6. Mages, H.W. *et al*. (2000) Eur. J. Immunol. **30**:1040.
7. Yoshinaga, S.K. *et al*. (1999) Nature **402**:827.

Flow Cytometry Validation

This antibody has been tested for flow cytometry using PHA-treated human peripheral blood cells.

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 this analysis, cells in a separate tube should be treated with PE-labeled mouse IgG_1 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.

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1-800-343-7475