PERFORMANCE DATA SHEET
1939

Monoclonal anti-human CD278(ICOS)/Biotin*

mAb name/Clone: **ANC6C6**
Isotype: Mouse IgG1κ
Immunogen: Human HPB-MLT cells, human ICOS-muIg fusion protein

CATALOG#: 265-030
QUANTITY: 100 µg **CONCENTRATION: 0.5 mg/ml**

INFORMATION: The inducible costimulator CD278 (ICOS, T cell activation molecule H4) is similar to human CD28 (24% homology), and plays an analogous role in the T cell activation process. Secondary signaling through CD28 or ICOS results in discrete cytokine secretion profiles by the activated T cells.[1] Signaling by either molecule is effectively down regulated by CD152 (CTLA-4) engagement.[2] Human CD275 (GL50, B7-H2) is a member of the B7 family sharing ~20% homology with CD80 (B7-1) and CD86 (B7-2), and has been shown to be a ligand for ICOS.[3] Two RNA splice variants exist for this molecule, differing only in the cytoplasmic domain. [4]
Antibody ANC6C6 binds to recombinant ICOS in EIA, and to the surface of stimulated PBL and HPB-MLT tumor cells in Flow cytometry. Additionally, it blocks binding of recombinant CD275-muIg to HPB-MLT cells.
References: 1) Beier, K.C., R.A. Kroczek, et al. 2000, *Eur J Immunol*. **30**(12):3707-3717. 2) Riley, J.L., C.H. June, et al. 2001, *J. Immunol.* **166**: 4943-4948. 3) Ling, V., M. Collins, et al. 2000, *J. Immunol.* **164**: 1653-1657. 4) Ling, V., M. Collins, et al. 2001, *J. Immunol.* **166**: 7300-7308.

STORAGE CONDITIONS: ***Store at 2 - 5°C***. Freeze/thawing not recommended.

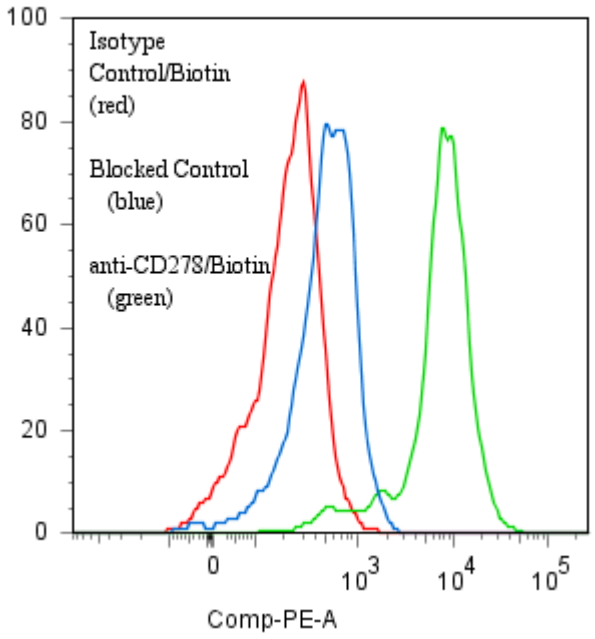
PRODUCT STABILITY: Product should retain activity for at least 12 months after shipping date when stored as recommended. Ship Date:_____

BUFFER: 50 mM Sodium Phosphate pH 7.5, 100 mM Potassium Chloride, 150mM NaCl, 5% Glycerol, 0.2% BSA, 0.04% NaN_3 (as a preservative).

PRODUCTION: Antibody from (low FBS containing) tissue culture supernatant was Protein A purified to >95% mouse immunoglobulin by SDS-PAGE (<1% bovine immunoglobulin), and reacted with NHS-Biotin. Unconjugated Biotin was removed from conjugate using a desalting column.

PERFORMANCE: Ficoll prepared human **peripheral blood mononuclear cells** were stimulated 5 days in culture with 5 µg/ml PHA after which they were harvested and washed in FACS buffer. Five x 10^5 cells per tube were pre incubated 5 minutes with 20 µl of 300 µg/ml human IgG (to block non specific binding) after which they were incubated 45 minutes on ice with 80 ml of anti-CD278/Biotin at **10 µg/ml**. Cells were washed twice and incubated with 2° reagent Streptavidin/R-PE (Catalog #253-050) after which they were washed three times, fixed and analyzed by FACS using a lymphoid gate. Cells stained positive with a mean shift of **1.53** $\log_{10}$ fluorescent units when compared to a Mouse IgG1/Biotin negative control (Catalog #278-030). Binding was blocked when cells were pre incubated 10 minutes with 20 µl of 0.5 mg/ml anti-CD278 antibody (Catalog #265-020).

Binding of anti-CD278(ICOS)/Biotin +SA/PE to stimulated human PBMC



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