PERFORMANCE DATA SHEET 3247 Monoclonal anti-human CD278(ICOS)/APC*

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

CATALOG#: 265-060 QUANTITY: 120 tests VOLUME IN VIAL: 0.2 ml WORKING DILUTION: 1:50 (or use 1.6μl of concentrated stock per 5 x 10⁵-cell test)

INFORMATION: The inducible costimulator (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 GL50 is a member of the B7 family sharing ~20% homology with CD80 (B7-1) and CD86 (B7-2), and has been shown to be the 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-muIg in EIA, and to the surface of HPB-MLT cells in Flow cytometry. Additionally, it blocks binding of recombinant GL50-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. Do not freeze! Protect from light.

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

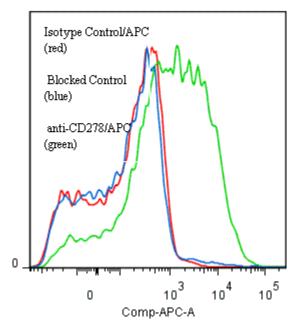
BUFFER: 50 mM Sodium Phosphate pH 7.5, 500 mM Potassium Chloride, 150mM NaCl, 15% Glycerol, 0.2% BSA, 0.04% NaN₃ (as a preservative).

PRODUCTION: Protein A purified antibody from tissue culture supernatant was conjugated to Allophycocyanin through a sulfo-ester linkage. Unconjugated antibody was removed using size exclusion chromatography. Antibody conjugate is at **0.5 mg/ml**, with an A_{650}/A_{280} ratio of 1.43.

PERFORMANCE: Five x 10^5 cultured human **HPB-MLT** cells were washed and incubated 45 minutes on ice with 80 µl of anti-CD278/APC at a **1:50** dilution (10 µg/ml). Cells were washed three times, fixed and analyzed by FACS. Cells stained positive with a mean shift of **1.09** log₁₀ fluorescent units when compared to a Mouse IgG1/APC negative control (Catalog #278-060). Binding was blocked when cells were pre incubated 10 minutes with 20 µl of 0.5 mg/ml unlabeled anti-CD278 antibody (Catalog #265-020).

*For Research use only. Not for use in Diagnostic Procedures

BInding of anti-CD278(ICOS)/APC to cultured human HPB-MLT T cell tumor line



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