

## PERFORMANCE DATA SHEET

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# Monoclonal anti-human CD278(ICOS)/APC\*

**mAb name/Clone:** ANC6C6

**Isotype:** Mouse IgG1 $\kappa$

**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 1.6 $\mu$ l of concentrated stock per 5 x 10<sup>5</sup>-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.<sup>1</sup> Signaling by either molecule is effectively down regulated by CD152 (CTLA-4) engagement.<sup>2</sup> 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.<sup>3</sup> Two RNA splice variants exist for this molecule, differing only in the cytoplasmic domain.<sup>4</sup>

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:** <sup>1</sup>) Beier, K.C., R.A. Kroczek, et al. 2000, *Eur J Immunol.* **30**(12):3707-3717. <sup>2</sup>) Riley, J.L., C.H. June, et al. 2001, *J. Immunol.* **166**: 4943-4948. <sup>3</sup>) Ling, V., M. Collins, et al. 2000, *J. Immunol.* **164**: 1653-1657. <sup>4</sup>) 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% NaN<sub>3</sub> (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<sub>650</sub>/A<sub>280</sub> ratio of 1.43.

**PERFORMANCE:** Five x 10<sup>5</sup> cultured human **HPB-MLT** cells were washed and incubated 45 minutes on ice with 80  $\mu$ l of anti-CD278/APC at a 1:50 dilution (10  $\mu$ g/ml). Cells were washed three times, fixed and analyzed by FACS. Cells stained positive with a mean shift of 1.09 log<sub>10</sub> 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  $\mu$ l 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

