PERFORMANCE DATA SHEET



Human ICOS-muIg/R-PE Fusion Protein*

CATALOG#: 517-050 **OUANTITY: 50 tests VOLUME IN VIAL: 0.16 ml WORKING DILUTION: 1:20** (or use 4 µl of concentrated stock per 5 x 10⁵-cell test)

Molecular Structure: A soluble molecule consisting of the extracellular (121aa) domain of mature human ICOS fused to

the murine IgG2a Fc (232 aa).

Transfectant Cell Line: CHO

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. Each secondary signal from CD28 or ICOS results in a discrete cytokine secretion profile displayed by the activated T cell. Both activation processes are 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 a ligand for ICOS.³ ICOSmuIg recombinant protein binds to recombinant GL50-muIg in EIA and also binds to cell surface ligand on Raji cells in Flow cytometry.

References: 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. ⁵ A.J. McAdam, et al. (2000) *J Immunol* **165**: 5035-5040.

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:

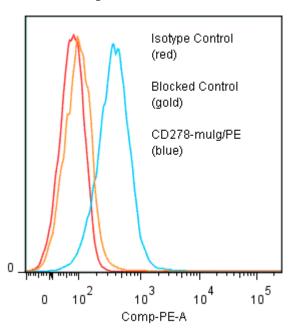
BUFFER: 50 mM Sodium Phosphate pH 7.5, 500 mM Potassium Chloride, 150mM NaCl, 15% Glycerol, 0.2% BSA, 0.04% NaN₃ (as a preservative).

PRODUCTION: Protein A purified recombinant protein from tissue culture supernatant was conjugated to R-Phycoerythrin through a sulfo-ester linkage. Unconjugated protein was removed using size exclusion chromatography. Protein conjugate is at **0.2 mg/ml** with an A_{565}/A_{280} ratio of 3.4.

PERFORMANCE: Five x 10⁵ cultured human **Raji** cells cells per tube were washed and incubated 45 minutes on ice with 80 µl of CD278(ICOS)-muIg/PE at a 1:20 dilution They were then washed twice, fixed and analyzed by FACS. Cells stained positive with a mean shift of log₁₀ fluorescent units when compared to a Mouse IgG2a/R-PE negative control (Catalog #281-050). Binding was blocked when reagent was pre incubated 10 minutes with a 5-fold excess of CD275(ICOSL)-muIg

* Research Use Only. Not for use in Diagnostic procedures. R-Phycoerythrin (R-PE) is covered under patents: U.S. 4,520,110; European 76,695 and Canadian 1,179,942.

Binding of CD278-mulg/PE to human Raji cells



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