

PERFORMANCE DATA SHEET

2605

Human CD154-muCD8/R-PE *Fusion Protein**

CATALOG#: 505-050

QUANTITY: 50 tests

VOLUME IN VIAL: 0.2 ml

WORKING DILUTION: 1:20 (or used 4 μ l of concentrated stock per 5×10^5 cell test)

Molecular Structure: A soluble fusion protein consisting of the extracellular domain (213aa) of human CD154 fused to the extracellular domain (167aa) of murine CD8 alpha.

Transfectant Cell Line: CHO

INFORMATION: Human CD154 (CD40 Ligand) is a member of the tumor necrosis factor (TNF) family and is expressed on the surface of activated T cells. Interaction of CD154 and CD40 is essential for isotype switching in B cells. Known genetic defects that alter this interaction lead to impaired immune system function (1). CD154 has been shown to be hyperexpressed by B and T cells in SLE patients (4). CD154 has been reported to be expressed on vascular endothelial cells, smooth muscle cells and macrophages indicating a possible role for the CD40-CD154 immunoregulatory signaling mechanism during inflammation and immunity in atherogenesis (6).

References: 1) D. Gray, et al, (1994) *Seminars in Immunol* 6: 303-310. 2) F. Pietravalle, et al, (1996) *J Biol Chemistry* 271: 5965-5967. 3) R.J. Noelle, (1996) *Immunity* 4: 415-419. 4) A. Desai-Mehta, et al, (1996) *J Clin Invest* 97: 2063-2073. 5) I.S. Grewal and R.A. Flavell, (1996) *Immunol Today* 17: 410-414. 6) F. Mach, et al, (1997) *Proc Natl Acad Sci USA* 94:1931-1936.

STORAGE CONDITIONS: Store at 2 - 5°C. Do not freeze! Protect from light.

PRODUCT STABILITY: Product should retain activity for at least 6 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: Purified fusion protein from tissue culture supernatant of transfectants 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 A₅₆₅/A₂₈₀ ratio of 2.3.

PERFORMANCE: Five $\times 10^5$ cultured human **Raji** cells per tube were washed and incubated 45 minutes on ice with 80 μ l of CD154-muCD8/R-PE at a 1:20 dilution (10 μ g/ml). [Alternatively, add 4 μ l of concentrated stock to cells in suspension.] Cells were washed twice, fixed and analyzed by FACS. Cells stained positive with a mean shift of 0.97 log₁₀ units when compared to a buffer control. Binding was blocked when reagent was pre incubated 30 minutes with anti-CD154 antibody (Catalog# 353-020) at a concentration of 100 μ g/ml.

*** Research Use Only. Not for use in Diagnostic procedures.**

Binding of CD154-muCD8/PE to human Raji cells

