PERFORMANCE DATA SHEET
2145

For maximal recovery of contents please quick spin vial before opening

Human CD154 (CD40 Ligand) muCD8 *Fusion Protein**

CATALOG#: 505-020
QUANTITY: 25 µg **CONCENTRATION: 0.5 mg/ml**

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. It can undergo proteolytic cleavage into a 19kD immunologically active soluble form. 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). Increased levels of CD154 has been associated with autoimmune disorders including SLE, CLL and eosinophilic fasciitis (5,9,10,11). CD154 has been reported to be expressed on vascular endothelial cells, smooth muscle cells, macrophages and activated platelets indicating a role for the CD40-CD154 immunoregulatory signaling in arthrosclerosis and cardiovascular disorders (7,12,13). Recombinant CD154-muCD8 has been shown to induce phosphorylation of ERK, JNK and p38 molecules and subsequent activation of NFkB pathway (14, 15). Human CD154-muCD8 binds to cell surface expressed human CD40 and this binding is blocked by anti-human CD154 monoclonal antibody.

REFERENCES: 1) D. Gray, et al, (1994) Seminars in Immunol 6: 303-310. **2)** A.C. Grammer, et al, (1995) J Immunol 154: 4996-5010. **3)** F. Pietravalle, et al, (1996) J Biol Chemistry 271: 5965-5967. **4)** R.J. Noelle, (1996) Immunity 4: 415-419. **5)** A. Desai-Mehta, et al, (1996) J Clin Invest 97: 2063-2073. **6)** I.S. Grewal and R.A. Flavell, (1996) Immunol Today 17: 410-414. **7)** F. Mach, et al, (1997) Proc Natl Acad Sci USA 94:1931-1936. **8)** A.C. Grammer, et al, (1999) J Immunol 163: 4150-4159. **9)** D. Hollenbaugh, (1992) *EMBO* 11: 4314-4321. **10)** R.K. Vakkalanka, et al, (1999) *Arthritis Rhem* 42:871-81. **11)** M. Jinnin, et al.(2003) *Ann Rhem Dis* 62: 190-191. **12)** U. Schonbeck, et al, (2000) *PNAS USA* 97: 7458-7463. **13)** U. Schonbeck, et al, (2001) *Circulation* 104: 2266-2268. **14)** AC Grammer, PF Lipsky, et al (2000) Arthritis Research and Therapy 76: 61-178. **15)** AC Grammer, PF Lipsky, et al (2004) Advances in Immunology 6: 28-38.

STORAGE CONDITIONS: ***Store at 2 - 5°C*. Do Not Freeze**.

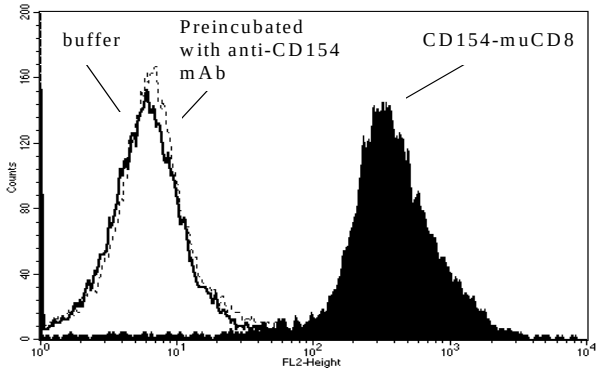
PRODUCT STABILITY: Product should retain activity for at least 6 months after shipping date when stored as recommended. Ship Date:_____

BUFFER: 50 mM Sodium Phosphate pH 7.5, 100 mM Potassium Chloride, 150mM NaCl, 0.5 mg/ml Gentamicin Sulfate (as a preservative).

PRODUCTION: Fusion protein from culture supernatant of CHO cell transfectants grown in protein free media was purified using size exclusion and affinity chromatography. Product was 0.1 µm filtered and vialed under aseptic conditions.

PERFORMANCE: Five x 10^5 cultured human **Raji** cells per tube were washed and incubated 45 minutes on ice with 80 µl of CD154-muCD8 at a concentration of **10 µg/ml**. Cells were washed twice and incubated with 50 µl of 2° reagent anti-mouse CD8α/R-PE (Catalog #260-050), after which they were washed three times, fixed and analyzed by FACS. Cells stained positive with a mean shift of **1.2** $\log_{10}$ fluorescent 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 µg/ml.

Binding of CD154-muCD8 to human Raji Cells



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