

## PERFORMANCE DATA SHEET

1818

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# 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 atherosclerosis 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 NFκB 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 Rheum* 42:871-81. **11)** M. Jinnin, et al.(2003) *Ann Rheum 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: \_\_\_\_\_

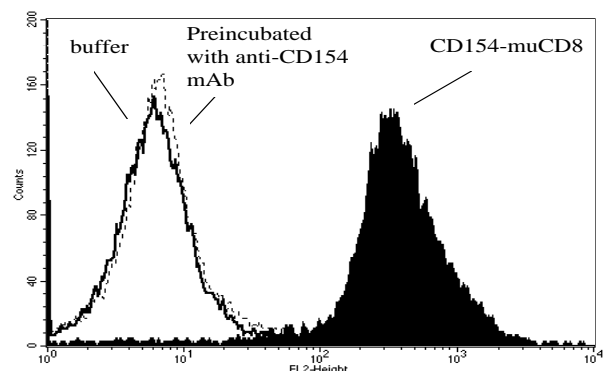
**BUFFER:** 50 mM Sodium Phosphate pH 7.5, 100 mM Potassium Chloride, 150mM NaCl, 0.5 mg/ml Gentamicin Sulfate (as a preservative).

**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 vialled under aseptic conditions.

**PERFORMANCE:** Five x 10<sup>5</sup> 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<sup>o</sup> 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<sub>10</sub>** fluorescent units when compared to a buffer control at a similar concentration. Binding was blocked when reagent was pre incubated 30 minutes with anti-CD154 antibody (Catalog #353-020) at a concentration of 100 µg/ml.

**\*This Product is intended for Laboratory Research use only.**

**Binding of CD154-muCD8 to human Raji Cells**



**Ancell Corporation P.O. Box 87 243 Third Street North Bayport, MN 55003-0087 USA**  
**Phone: Toll free 800-374-9523 or 651-439-0835 Fax: 651-439-1940**