

PERFORMANCE DATA SHEET

2606

Monoclonal anti-human CD154 (CD40 Ligand)/R-PE*

mAb name/Clone: 24-31

Isotype: Murine IgG1

Immunogen: Human sgp39 fusion protein

CATALOG#: 353-050

QUANTITY: 120 tests

VOLUME IN VIAL: 0.2 ml

WORKING DILUTION: 1:50 (or use 1.6µl of concentrated stock per 5 x 10⁵-cell test)

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 an 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). Antibody 24-31 immunoprecipitates a CD154 (gp39) molecule of about 39 kd. The antibody 24-31 will block MLR, sgp39 induced human B cell proliferation and T cell dependent B cell differentiation.

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.

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% Na₃ (as a preservative).

PRODUCTION: Protein A purified antibody from tissue culture supernatant was conjugated to R-Phycoerythrin through a sulfo-ester linkage. Unconjugated antibody was removed using size exclusion chromatography. Antibody conjugate is at 0.5 mg/ml with an A₅₆₅/A₂₈₀ ratio of 2.8.

PERFORMANCE: Ficoll prepared human PBMC were stimulated in culture 6 hours at 5 x 10⁶ cells/ml in RPMI 10% FBS media including 1 µM Ionomycin and 10 ng/ml Phorbol 12-Myristate 13-Acetate (PMA). Five x 10⁵ cells per tube were then washed and incubated 45 minutes on ice with 80 µl of anti-CD154/R-PE at a 1:50 dilution (10 µg/ml). Cells were washed three times, fixed and analyzed by FACS using a lymphoid gate. A 51% sub population of the cells stained positive with a mean shift of 1.82 log₁₀ fluorescent units when compared to a Mouse IgG1/R-PE negative control (Catalog #278-050). Binding was blocked when cells were pre incubated 10 minutes with 20 µl of 0.5 mg/ml unlabeled anti-CD154 antibody (Catalog #353-020).

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

Binding of anti-CD154/PE to stimulated human PBL

