

PERFORMANCE DATA SHEET

1818

Monoclonal anti-human CD154 (CD40 Ligand)/FITC*

mAb name/Clone: 24-31

Isotype: Murine IgG1

Immunogen: Human sgp39 fusion protein

CATALOG#: 353-040

QUANTITY: 120 tests

VOLUME IN VIAL: 0.2ml

WORKING DILUTION: 1:50 (or use 1.6µl of concentrated stock per 5×10^5 -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. Freeze/Thawing is not recommended. Protect from light.

PRODUCT STABILITY: Product should retain activity for at least 12 months after shipping date when stored as recommended. Ship Date: _____

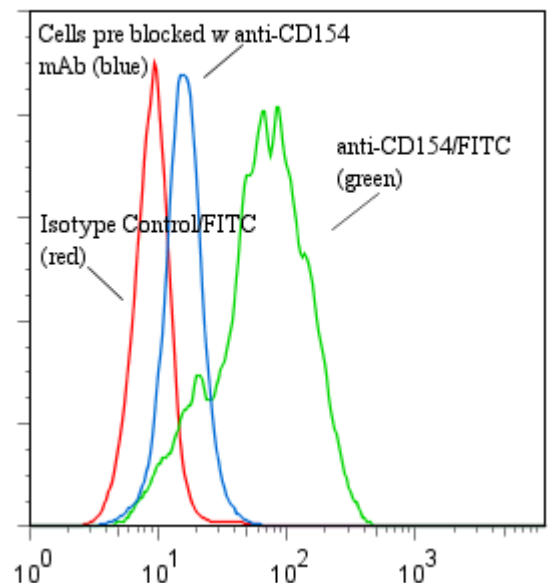
BUFFER: 50 mM Sodium Phosphate pH 7.5, 100 mM Potassium Chloride, 150mM NaCl, 5% Glycerol, 0.2% BSA, 0.04% NaN₃ (as a preservative).

PRODUCTION: Protein A purified antibody from tissue culture supernatant was reacted with FITC. Unconjugated FITC was separated from antibody/FITC conjugate by desalting column. The antibody/FITC conjugate is at 0.5 mg/ml with a Fluorescein/IgG molar ratio of 10.5.

PERFORMANCE: Cultured human Jurkat-4G cells (a CD154⁺ subclone of Jurkat) were stimulated by incubating 6 hours at 5×10^6 cells/ml in RPMI 10% FBS media including 1 µM Ionomycin and 10 ng/ml Phorbol 12-Myristate 13-Acetate. Five $\times 10^5$ cells per tube were washed and pre incubated 5 minutes with 20 µl of 250 µg/ml human IgG (to block non specific binding, after which they were incubated 45 minutes on ice with 80 µl of anti-CD154/FITC at a 1:50 dilution factor (10µg/ml). Cells were then washed three times, fixed and analyzed by FACS. Cells stained positive with a mean shift of 0.9 log₁₀ fluorescent units when compared to a Mouse IgG1/FITC negative control (Catalog #278-040) at a similar concentration. 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).

*This Product is intended for Laboratory Research use only.

Binding of anti-CD154/FITC to stimulated human Jurkat-4G 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