

# PERFORMANCE DATA SHEET

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## Monoclonal anti- human CD154 (CD40 Ligand)/Biotin\*

**mAb name/Clone:** 24-31

**Isotype:** Murine IgG1

**Immunogen:** Human sgp39 fusion protein

**CATALOG#:** 353-030

**QUANTITY:** 100 µg

**CONCENTRATION:** 1.0 mg/ml

**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 not recommended.

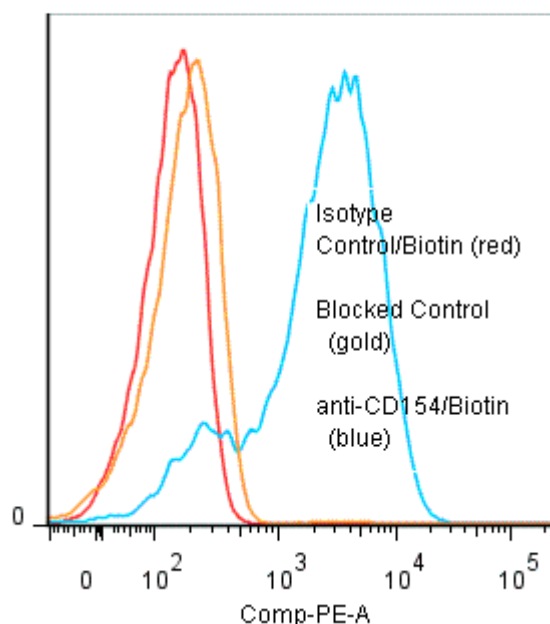
**PRODUCT STABILITY:** Product should retain activity for at least 12 months after shipping date when stored as recommended. Ship Date: \_\_\_\_\_

**BUFFER:** 50 mM Sodium Phosphate pH 7.5, 100 mM Potassium Chloride, 150mM NaCl, 5% Glycerol, 0.2% BSA, 0.04% NaN<sub>3</sub> (as a preservative).

**PRODUCTION:** Antibody from (low FBS containing) tissue culture supernatant was Protein A purified to >95% mouse immunoglobulin by SDS-PAGE (<1% bovine immunoglobulin), and reacted with NHS-Biotin. Unconjugated Biotin was removed from conjugate using a desalting column.

**PERFORMANCE:** Human **Jurkat-4G** cells (a CD154 positive subclone of Jurkat) were stimulated 6 hours in the presence of 10 ng/ml PMA and 1 µM Ionomycin. Cells were harvested and washed. Five x 10<sup>5</sup> cells per tube were washed and incubated 45 minutes on ice with 80 µl of anti-CD154/Biotin at **10 µg/ml**. Cells were washed twice and incubated with 2<sup>o</sup> reagent Streptavidin/R-PE (Catalog #253-050) after which they were washed three times, fixed and analyzed by FACS. A subpopulation of the cells stained positive with a mean shift of 1.35 log<sub>10</sub> fluorescent units when compared to a Mouse IgG1/Biotin negative control (Catalog #278-030). Binding was blocked when cells were pre incubated 10 minutes with 20 µl of 0.5 mg/ml anti-CD154 antibody (Catalog #353-020).

### Blinding of anti-CD154/Biotin +SA/PE to stimulated human Jurkat-4G cells



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