

# PERFORMANCE DATA SHEET

2523

## 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 per 5 x 10<sup>5</sup>-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. 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. CD154 has been shown to be hyperexpressed by B and T cells in SLE patients. 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:** D. Gray, et al, (1994) Seminars in Immunol **6**: 303-310. F. Pietravalle, et al, (1996) J Biol Chemistry **271**: 5965-5967. S.M.A. Lens, (1996) J Immunol **156**: 507-514. R.J. Noelle, (1996) Immunity **4**: 415-419. A. Desai-Mehta, et al, (1996) J Clin Invest **97**: 2063-2073. I.S. Grewal and R.A. Flavell, (1996) Immunol Today **17**: 410-414. K.M. Aagaard-Tillery and D.F. Jelinek, (1996) J Immunol **157**: 2769-2778.

**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% Na<sub>3</sub> (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 8.2.

**PERFORMANCE:** Ficoll prepared human **PBMC** were stimulated in culture by incubating 6 hours at 5 x 10<sup>6</sup> cells/ml in RPMI 10% FBS media including 1 µM Ionomycin and 10 ng/ml Phorbol 12-Myristate 13-Acetate. Five x 10<sup>5</sup> 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 (10 µg/ml). Cells were then washed three times, fixed and analyzed by FACS. A net **60%** sub population of the cells stained positive with a mean shift of **1.48 log<sub>10</sub>** fluorescent units when compared to a Mouse IgG1/FITC negative control (Catalog # 278-040). Binding was blocked when cells were pre incubated 10 minutes with 20 µl of 0.5 mg/ml anti-CD154 antibody (Catalog #353-020).

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

**Binding of anti-CD154/FITC to stimulated human PBL**

