## PERFORMANCE DATA SHEET

3032

## Human CD40 muIg/R-PE Fusion Protein\*



CATALOG#: 504-050
QUANTITY: 50 tests
VOLUME IN VIAL: 0.2 ml
WORKING DILUTION: 1:20 (or use 4 ul of concentrated stock per 5 x 10<sup>5</sup>-cell test)

Molecular Structure: A soluble fusion protein consisting of the extracellular (193aa) domain of human

CD40 fused to murine IgG2a Fc (233aa).

Transfectant Cell Line: CHO

**INFORMATION:** Human CD40 is a member of the tumor necrosis factor (TNF) receptor family and is present on all B cells except plasma cells. CD40 is also found on some epithelial cells, carcinomas and lymphoid dendritic cells. CD40 plays an important role in B cell activation and the interaction with its ligand CD154 (CD40 Ligand) is essential for isotype switching (1). CD40 muIg fusion protein is recognized by anti-human CD40 antibody (catalog #300-020) and binds to human CD154. This binding can be blocked by anti-CD154 antibody (catalog #353-020). **References:** (1) T.A. Foy, et al, (1996) Annu Rev Immunol 14: 591-617. (2) M.E. Ozaki, et al, (1997) J Immunol 159: 214-221.

STORAGE CONDITIONS: Store at 2 - 5°C. Do not freeze! Protect from light.

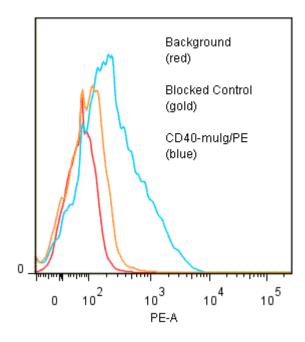
**PRODUCT STABILITY:** Product should retain activity for at least 12 months after shipping date when stored as recommended. Ship Date:\_\_\_\_\_

**BUFFER:** 50 mM Sodium Phosphate pH 7.5, 500 mM Potassium Chloride, 150mM NaCl, 15% Glycerol, 0.2% BSA, 0.04% NaN<sub>3</sub> (as a preservative).

**PRODUCTION:** Protein A purified recombinant protein from tissue culture supernatant was conjugated to R-Phycoerythrin through a sulfo-ester linkage. Unconjugated protein was removed using size exclusion chromatography. Protein conjugate is at a concentration of **0.4 mg/ml** with an  $A_{565}/A_{280}$  ratio of **3.59**.

**PERFORMANCE:** Ficoll prepared human **peripheral blood mononuclear cells** were stimulated 6 hours in culture in the presence of 10 ng/ml PMA and 1 $\mu$ M Ionomicin to upregulate CD154 expression. Five x 10<sup>5</sup> cells per tube were washed and incubated 45 minutes on ice with 80 ul of a **1:20** dilution (20  $\mu$ g/ml) of CD40-muIg/R-PE. They were then washed three times, fixed and analyzed by FACS. A **42% Sub population** of the cells stained positive with a mean shift of **1.09**  $\log_{10}$  fluorescent units when compared to a negative control. Binding was blocked when cells were pre incubated 10 minutes with 20  $\mu$ l of 0.5 mg/ml anti-CD154 antibody (clone 24-31; Catalog #353-020).

## Binding of CD40-mulg/PE to stimulated human PBL



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<sup>\*</sup> Research Use Only. Not for use in Diagnostic procedures.