

# PERFORMANCE DATA SHEET

3303

## 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  $5 \times 10^5$ -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.**

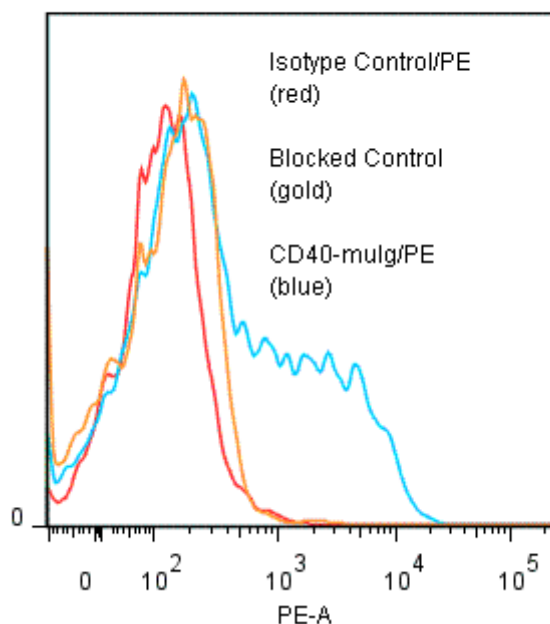
**PRODUCT STABILITY:** Product should retain activity for at least 12 months after shipping date when stored as recommended. Ship Date: \_\_\_\_\_

**BUFFER:** 50 mM Sodium Phosphate pH 7.5, 500 mM Potassium Chloride, 150mM NaCl, 15% Glycerol, 0.2% BSA, 0.04%  $\text{NaN}_3$  (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 0.2 mg/ml with an  $A_{565}/A_{280}$  ratio of 4.0.

**PERFORMANCE:** Ficoll prepared human peripheral blood mononuclear cells were stimulated 6 hours in culture in the presence of 10 ng/ml PMA and 1  $\mu\text{M}$  Ionomycin to upregulate CD154 expression. Five  $\times 10^5$  cells per tube were washed and incubated 45 minutes on ice with 80 ul of a 1:20 dilution (10  $\mu\text{g}/\text{ml}$ ) of CD40-muIg/R-PE. They were then washed three times, fixed and analyzed by FACS using a lymphoid gate. A net 29% sub population of the cells stained positive with a mean shift of 1.41  $\log_{10}$  fluorescent units when compared to a negative control. Binding was blocked when cells were pre incubated 10 minutes with 20  $\mu\text{l}$  of 0.5 mg/ml anti-CD154 antibody (clone 24-31 Catalog #353-020).

### CD40-muIg/PE binding to stimulated human PBL



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