

PERFORMANCE DATA SHEET

3240

Monoclonal anti-human CD28/FITC*

mAb name/Clone: ANC28.1/5D10

Isotype: Mouse IgG1κ

Immunogen: Human CD28 Ig fusion protein and HPB-MLT human tumor cells

CATALOG#: 177-040

QUANTITY: 120 tests

VOLUME IN VIAL: 0.2ml

WORKING DILUTION: 1:50 (or use 1.6µl of concentrated stock per 5 x 10⁵-cell test)

INFORMATION: Human CD28 is an important costimulatory molecule found on all CD4+ T cells and on about half of the CD8+ T cells. T cell activities attributed to CD28 include prevention of anergy, induction of cytokine gene transcription, stabilization of cytokine mRNA and activation of CD8+ cytotoxic T lymphocytes. The ligands for CD28 identified as CD80(B7-1) and CD86(B7-2), are immunoglobulin superfamily monomeric transmembrane glycoproteins of 60 kd and 80 kd respectively. Anti-CD28 antibodies have recently been shown to have a strong adjuvant effect when conjugated to the immunogen (4). Antibody ANC28.1 recognizes CD28 expressed on human T cells and also on BHK cells transfected with human CD28. Monomeric and cross linked ANC28.1 will stimulate expression of IL-2 from CD28⁺ cells.

References: (1) M.K. Jenkins & J.G. Johnson, (1993) *Curr Opin Immunol* 5: 361-367. (2) 61st Forum in Immunology, (1995) *Res Immunol* 146: 127-205. (3) I. Kariv, A. Truneh & R.W. Sweet, (1996) *J Immunol* 157: 29-38. (4) J. Carling, et al, (2003) *J Immunol* 33: 135-142.

STORAGE CONDITIONS: Store at 2 - 5°C. Freeze/Thawing is not recommended. **Protect from light.**

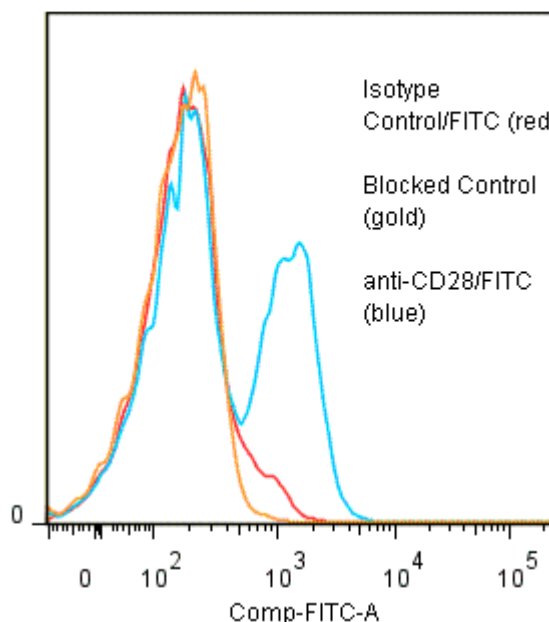
PRODUCT STABILITY: Product should retain activity for at least 12 months after shipping date when stored as recommended. Ship Date: _____

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 0.25 mg/ml with a Fluorescein/IgG molar ratio of 5.3.

PERFORMANCE: Five x 10⁵ ficoll prepared human PBMC per tube were washed and pre incubated 5 minutes with 20 µl human IgG at 250 µg/ml (to block non specific binding) after which they were incubated 45 minutes on ice with 80 µl of anti-CD28/FITC at a 1:50 dilution (5 µg/ml). Cells were washed three times, fixed and analyzed by FACS. A 36% sub population of the cells stained positive with a mean shift of 0.8 log₁₀ 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-CD28 antibody (Catalog #177-020).

Binding of anti-CD28/FITC to human PBL



* **Research use only. Not for use in Diagnostic procedures.**