

**PERFORMANCE DATA SHEET**

2732

**Monoclonal anti-human CD28/R-PE \***

**mAb name/Clone:** ANC28.1/5D10

**Isotype:** Mouse IgG1κ

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

**CATALOG#:** 177-050

**QUANTITY:** 120 tests

**VOLUME IN VIAL:** 0.2 ml

**WORKING DILUTION:** 1:50 (or use 1.6µl of concentrated stock per 5 x 10<sup>5</sup>-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<sup>+</sup> 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. 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 antibody from tissue culture supernatant was conjugated to R-Phycoerythrin through a sulfo-ester linkage. Unconjugated antibody was removed using size exclusion chromatography. Antibody conjugate is at **0.5 mg/ml** with an A<sub>565</sub>/A<sub>280</sub> ratio of 2.65.

**PERFORMANCE:** Five x 10<sup>5</sup> cultured **HPB-MLT** cells were washed and incubated 45 minutes on ice with 80 µl of anti-CD28/R-PE at a **1:50** dilution (10µg/ml). Cells were washed three times, fixed and analyzed by FACS. Cells stained positive with a mean shift of **2.03** log<sub>10</sub> fluorescent units when compared to a Mouse IgG1/R-PE negative control (Catalog #278-050). Binding was blocked when cells were pre incubated 10 minutes with 20 µl of 0.5 mg/ml anti-CD28 antibody (Catalog #177-020).

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

**Binding of anti-CD28/PE to human HPB-MLT cells**

