

PERFORMANCE DATA SHEET

2519

Monoclonal anti-human CD28/Biotin* (stimulating)

mAb name/Clone: ANC28.1/5D10

Isotype: Mouse IgG1κ

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

CATALOG#: 177-030

QUANTITY: 100 µg

CONCENTRATION: 1.0 mg/ml

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 not recommended.

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: Antibody from (low FBS containing) tissue culture supernatant was Protein A purified to >95% mouse immunoglobulin by SDS-PAGE (<1% bovine immunoglobulin), and reacted with NHS-Biotin. Unconjugated Biotin was removed from conjugate using a desalting column.

PERFORMANCE: Five x 10⁵ cultured HPB-MLT cells were washed and incubated 45 minutes on ice with 80 µl of anti-CD28/Biotin at 5 µg/ml. Cells were washed twice and incubated with 2^o reagent Streptavidin/R-Phycoerythrin (Catalog #253-050), after which they were washed three times, fixed and analyzed by FACS. Cells stained positive with a mean shift of 1.88 log₁₀ fluorescent units when compared to a Mouse IgG1/Biotin negative control (Catalog #278-030) at a similar concentration. 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/Biotin +SA/PE to HPB-MLT cells

