

PERFORMANCE DATA SHEET

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Monoclonal anti-human CD357 (GITR,AITR)/FITC* (IgM isotype)

mAb name/Clone: ANC7D6-1B7

Isotype: Mouse IgMκ

Immunogen: Recombinant human GITR, PHA stimulated human PBL

CATALOG#: 268-040

QUANTITY: 120 tests

VOLUME IN VIAL: 0.2 ml

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

INFORMATION: Glucocorticoid induced Tumor Necrosis Factor receptor family related gene (CD357, GITR, AITR) is a type 1 228 amino acid transmembrane protein that has been designated TNFRSF18. GITR is expressed mainly by CD4+ CD25+ T lymphocytes. Its engagement by antibodies or its ligand (GITRL) induces a costimulatory signal which can break T cell suppression (2). GITR has been implicated as a regulatory mechanism in anti-parasitic immune responses (4), and is involved with Macrophages activation in atherosclerotic plaque formation (3).

Clone ANC7D6-1E7 binds to human CD357 in EIA and on stimulated cell surface in FACS. Pre incubation of the receptor with an excess of this mAb will block binding of recombinant GITR Ligand.

References: 1) Gurney AL, et al. (1999) *Curr Biol* **9**(4): 215-218. 2) Ji HB, et al. (2004) *J Immunol* **172**(10): 5823-5827. 3) Kim WJ, WH Lee, et al. (2006) *Immunology* **119**(3): 44-9. 4) Furze RC, Selkirk ME, et al. (2006) *Microbes Infect* **8**(12-13): 2803-10. 5) Kohm AP, SD Miller, et al. (2004) *J Immunol* **172**: 4686-4690. 6) McHugh, R.S. et al. (2002) *Immunity* **16**:311. 7) Shin, H.H. et al. (2002) *FEBS Lett.* **514**:275. 8). Shimizu, J. et al. (2002) *Nature Immunol.* **3**:135.

STORAGE CONDITIONS: Store at 2 - 5°C. Freeze thawing 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, 500 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 purified by size exclusion chromatography, and reacted with FITC. Unconjugated FITC was removed from conjugate using a desalting column. Antibody conjugate is at **0.5 mg/ml** with a Fluorescein/IgM molar ratio of 35.

PERFORMANCE: Ficoll prepared human **peripheral blood mononuclear cells** (PBMC) were stimulated 5 days in culture with PHA. Five x 10⁵ cells per tube were washed and pre incubated 5 minutes with 20 µl of 250 µg/ml human IgG (to block non specific binding) after which they were incubated 45 minutes on ice with 80 µl of anti-GITR/FITC at a 1:50 dilution factor (10µg/ml). Cells were washed three times, fixed and analyzed by FACS. Cells stained positive with a mean shift of **1.03 log₁₀** fluorescent units when compared to a Mouse IgM/FITC negative control (Catalog #290-040) at a similar concentration. Binding was blocked when cells were pre incubated with a 10-fold excess of unlabeled anti-GITR mAb (Catalog #268-020).

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

Binding of anti-GITR/FITC to stimulated human PBMC

