

PERFORMANCE DATA SHEET

2539

Monoclonal anti-human CD357 (GITR, AITR)/R-PE *

mAb name/Clone: ANC5A3

Isotype: Mouse IgG3κ

Immunogen: Recombinant human GITR, PHA stimulated human PBL

CATALOG#: 368-050

QUANTITY: 120 tests

VOLUME IN VIAL: 0.2 ml

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

INFORMATION: Glucocorticoid induced Tumor Necrosis Factor receptor family related gene (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 ANC5A3 binds to human GITR in EIA and on stimulated cell surface in FACS. Preincubation 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. 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% NaN₃ (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 **0.75 mg/ml**, with an A₅₆₅/A₂₈₀ ratio of 2.7.

PERFORMANCE: Ficoll prepared human **peripheral blood leukocytes** (PBL) 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/R-PE at a **1:50** dilution (15µg/ml). Cells were washed three times and analyzed by FACS. Cells stained positive with a mean shift of **1.33** log₁₀ fluorescent units when compared to a Mouse IgG3/R-PE negative control (Catalog #287-050). Binding was blocked when cells were pre incubated with a 10-fold excess of unlabeled anti-GITR mAb (cat #368-020).

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

Binding of anti-CD357(GITR)/PE to stimulated human PBL

