## PERFORMANCE DATA SHEET



## Monoclonal anti-human IFN-gamma/FITC\*

mAb name/Clone: ANC2E11 *Isotype:* Mouse IgG1κ

Immunogen: Recombinant human IFN-gamma

CATALOG#: 247-040 **VOLUME IN VIAL: 0.2ml QUANTITY: 120 tests WORKING DILUTION: 1:50** (or use 1.6ml of concentrated stock per 5 x 10<sup>5</sup>-cell test)

**INFORMATION:** The cytokine human interferon-gamma (IFN-gamma) is a glycosylated protein that exists as a homodimer of 34 kd in its biologically active form. IFN-gamma is expressed upon activation by TH1 cells CD8<sup>+</sup> cells and NK cells (2). Secreted IFN-gamma is a potent inhibitor of in vitro hematopoiesis and appears to serve as an important regulator of lymphocyte and macrophage function by exerting antiproliferative effects (1). Antibody ANC2E11 binds to recombinant and cellular IFN-gamma in EIA and Flow cytometry.

References: 1) H.A. Young & K.J. Hardy (1995) J Leuk Biol 58:373-381. 2) A. Bilkiau (1996) Adv Immunol 62:61-130. 3) T. Sato, et al (1997) Blood 90:4749-4758.

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

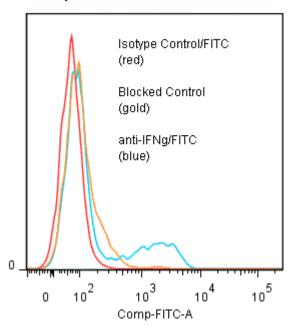
**PRODUCT STABILITY:** Product should retain activity for at least 12 months after shipping date when stored as recommended. Ship Date:

BUFFER: 50 mM Sodium Phosphate pH 7.5, 100 mM Potassium Chloride, 150mM NaCl, 5% Glycerol, 0.2% BSA, 0.04% NaN<sub>3</sub> (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 0.1 mg/ml with a Fluorescein/IgG molar ratio of 8.1.

PERFORMANCE: Ficoll prepared human peripheral blood lymphocytes were stimulated 4 hours in the presence of 50 ng/ml PMA, 2 µM ionomicin and 5µM monensin. Cells were then harvested, washed and fixed for 30 min with 2% buffered formaldehyde after which they were washed two times. Fixed cells were permeabolized 10 minutes in a buffer containing 0.05% Saponin. Subsequent reagent incubations and washes were done using this buffer. Five  $x 10^5$  cells per tube were preincubated 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-IFN-gamma/FITC at a 1:50 dilution factor (2 µg/ml). Cells were washed three times, fixed and analyzed by FACS. A net 21% sub population of the cells stained positive with a mean shift of 1.5 log<sub>10</sub> 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-IFN-gamma antibody (Catalog #247-020).

## Binding of anti-IFNg/FITC to stimulated fixed permeabolized human PBMC



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<sup>\*</sup>Research use only. Not for use in Diagnostic procedures.