

PERFORMANCE DATA SHEET

1817

*Monoclonal anti-human IFN-gamma/R-PE**

mAb name/Clone: ANC2E11

Isotype: Mouse IgG1 κ

Immunogen: Recombinant human IFN-gamma

CATALOG#: 247-050

QUANTITY: 120 tests

VOLUME IN VIAL: 0.2 ml

WORKING DILUTION: 1:50 (or use 1.6 μ l of concentrated stock per 5 x 10⁵-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⁺ 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. Bilkiu (1996) Adv Immunol 62:61-130. 3) T. Sato, et al (1997) Blood 90:4749-4758.

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 **250 μ g/ml** with an A₅₆₅/A₂₈₀ ratio of 3.69.

PERFORMANCE: Ficoll prepared human **peripheral blood lymphocytes** were stimulated 4 hours in the presence of 50 ng/ml PMA, 2 μ M ionomycin and 5 μ M 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 permeabilized 10 minutes in a buffer containing **0.3% Saponin**. Subsequent reagent incubations and washes were done using this buffer. Five x 10⁵ cells per tube were preincubated 5 minutes with 20 μ l of 250 μ g/ml human IgG (to block nonspecific binding), after which they were incubated 45 minutes on ice with 80 μ l of anti-IFN-gamma/FITC at a **1:50** dilution factor (5 μ g/ml). Cells were washed three times, fixed and analyzed by FACS. A net **9%** sub population of the cells stained positive with a mean shift of **1.1 log₁₀** fluorescent units when compared to a Mouse IgG1/R-PE negative control (Catalog # 278-050) at a similar concentration. Binding was 90% blocked when cells were pre incubated 10 minutes with 20 μ l of 0.5 mg/ml anti-IFNgamma antibody (Catalog #247-020).

***This Product is intended for Laboratory Research use only.** R-Phycoerythrin (R-PE) is covered under patents: U.S. 4,520,110; European 76,695 and Canadian 1,179,942.