

# PERFORMANCE DATA SHEET

2919

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

**mAb name/Clone:** ANC2E11

**Isotype:** Mouse IgG1 $\kappa$

**Immunogen:** Recombinant human IFN-gamma

**CATALOG#:** 247-030

**QUANTITY:** 100  $\mu$ g

**CONCENTRATION:** 1.0 mg/ml

**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 not recommended.

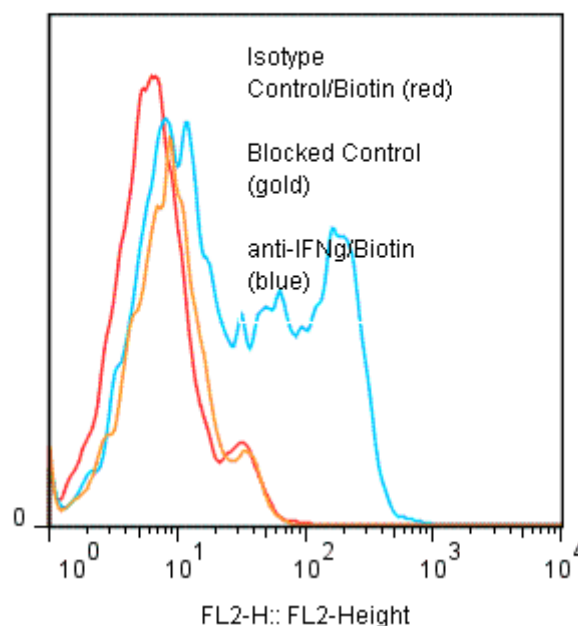
**PRODUCT STABILITY:** Product should retain activity for at least 12 months after shipping date when stored as recommended. Ship Date:\_\_\_\_\_

**BUFFER:** 50 mM Sodium Phosphate pH 7.5, 100 mM Potassium Chloride, 150mM NaCl, 5% Glycerol, 0.2% BSA, 0.04% NaN<sub>3</sub> (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:** Ficoll prepared human peripheral blood lymphocytes were stimulated 4 hours in the presence of 50 ng/ml PMA, 2  $\mu$ M ionomycin and 5  $\mu$ 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 10<sup>5</sup> cells per tube were preincubated 5 minutes with 20  $\mu$ l of 250  $\mu$ g/ml human IgG (To block nonspecific binding), after which they were incubated 45 minutes on ice with 80  $\mu$ l of anti-IFN gamma/Biotin at 1  $\mu$ g/ml. Cells were washed twice and incubated with 2<sup>o</sup> reagent Streptavidin/R-PE (Catalog #253-050) after which they were washed three times, fixed and analyzed by FACS. A net 40% sub population of the cells stained positive with a mean shift of 1.0 log<sub>10</sub> fluorescent units when compared to a Mouse IgG1/Biotin negative control (Catalog # 278-030). Binding was blocked when cells were pre incubated 10 minutes with 20  $\mu$ l of 0.5 mg/ml anti-IFN-gamma antibody (Catalog #397-020).

### Binding of anti-IFN $\gamma$ /Biotin +SA/PE to fixed permeabilized stimulated human PBMC



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