

PERFORMANCE DATA SHEET

1818

Monoclonal anti- human TNF alpha/FITC*

mAb name/Clone: J1D9

Isotype: Mouse IgG1

Immunogen: Recombinant TNF alpha

CATALOG#: 398-040

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: Human tumor necrosis factor alpha (TNF alpha) is a proinflammatory cytokine expressed mainly by activated monocytes and macrophages. TNF alpha exerts a key role with regard to pathogenesis of many infectious and inflammatory diseases. Antibody J1D9 neutralizes TNF alpha biological activities (2).

References: 1) P. Vassalli, (1992) Annu Rev Immunol 10: 411-452. 2) P.J. McLaughlin, et al, (1992) Anticancer Res 12: 1243-1246. 3) M. Pasparakis, et al, (1996) Cytokine and Growth Factor Reviews 7: 223-229. 4) A. Eigler, et al, (1997) Immunology Today 18: 487-492.

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% NaN₃ (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 8.4 .

PERFORMANCE: Ficoll prepared human peripheral blood mononuclear cells were stimulated by culturing two days with 5 µg/ml PHA-P, 1 µg/ml anti-CD28 antibody (cat# 177-820), and 1 µg/ml Brefeldin A. 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 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-TNFα/FITC at a 1:50 dilution (2 µg/ml). Cells were washed three times, fixed and analyzed by FACS. A net 3.9% sub population of the cells stained positive with a mean shift of 1.21 log₁₀ fluorescent units when compared to a Mouse IgG1/FITC negative control (Catalog #278-040) at a similar concentration. Binding was blocked when cells were pre incubated 10 minutes with 20 µl of 0.5 mg/ml anti-TNFα antibody (Catalog #398-020).

**This Product is intended for Laboratory Research use only.*

Binding of anti-TNF alpha/FITC to fixed permeabilized stimulated human PBMC

