

# PERFORMANCE DATA SHEET

1818

## Monoclonal anti-human TNF alpha/Biotin\*

**mAb name/Clone:** J1D9

**Isotype:** Mouse IgG1

**Immunogen:** Recombinant TNF alpha

**CATALOG#:** 398-030

**QUANTITY:** 100 µg

**CONCENTRATION:** 1.0 mg/ml

**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.

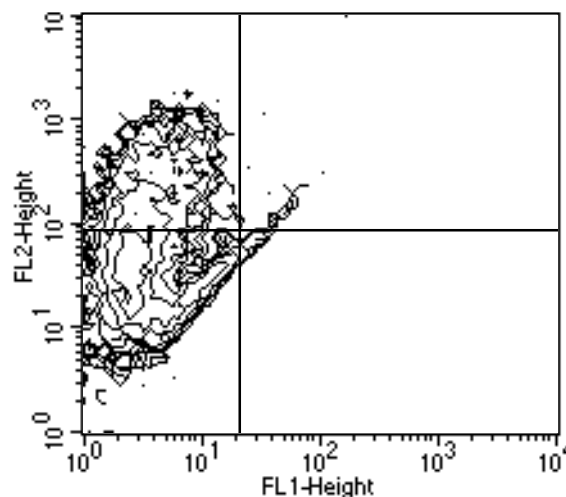
**STORAGE CONDITIONS:** Store at 2 - 5°C. Freeze/thawing not recommended.

**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 mononuclear cells** were stimulated by incubating 6 hours in the presence of 50 ng/ml PMA, 1 µM ionomycin 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 10<sup>5</sup> 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/Biotin at **1.0 µg/ml**. Cells were washed twice and incubated 20 µl of 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 **32%** population of the cells stained positive with a mean shift of **1.06 log<sub>10</sub>** fluorescent units when compared to a Mouse IgG1/Biotin negative control (Catalog #278-030). Binding was blocked when pre incubated 10 minutes with 20 µl of 0.5 mg/ml anti-TNFα antibody (Catalog #398-020).



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