## PERFORMANCE DATA SHEET <sup>1818</sup> *Monoclonal* anti-human TNF alpha/R-PE\*

*mAb name/Clone:* J1D9 *Isotype:* Mouse IgG1 *Immunogen:* Recombinant TNF alpha

CATALOG#: 398-050 QUANTITY: 120 tests VOLUME IN VIAL: 0.2 ml WORKING DILUTION: 1:50 (or use 1.6μl of concentrated stock per 5 x 10<sup>5</sup>-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. 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% NaN<sub>3</sub> (as a preservative).

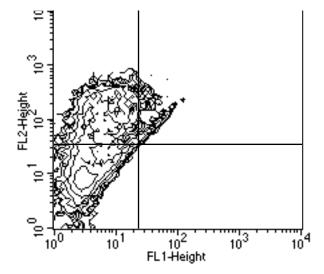
**PRODUCTION:** Protein A purified antibody from tissue culture supernatant was conjugated to R-Phycoerythrin through a sulfo-ester linkage. Unconjugated antibody was removed using size exclusion chromatography. Antibody conjugate is at  $250\mu g/ml$  with an  $A_{565}/A_{280}$  ratio of 3.21.

**PERFORMANCE:** Ficoll prepared human **peripheral blood mononuclear cells** were stimulated by incubating 6 hours in the presence of 50 ng/ml PMA, 1  $\mu$ M ionomycin and 1  $\mu$ g/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 permeabolized 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  $\mu$ l of 250  $\mu$ g/ml human IgG (to block non specific binding) after which they

were incubated 45 minutes on ice with 80  $\mu$ l of anti-TNF $\alpha$ /R-PE at a **1:50 dilution** (5  $\mu$ g/ml). Cells were washed three times, fixed and analyzed by FACS. A net **27%** sub population of the cells stained positive with a mean shift of **0.85** log<sub>10</sub> fluorescent units when compared to a Mouse IgG1/R-PE negative control (Catalog #278-050) at a similar concentration. Binding was blocked when cells were pre incubated 10 minutes with 20  $\mu$ l of 0.5mg/ml anti-TNF $\alpha$  antibody (Catalog #398-020).

\**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.



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