PERFORMANCE DATA SHEET
3039

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 10^5-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.

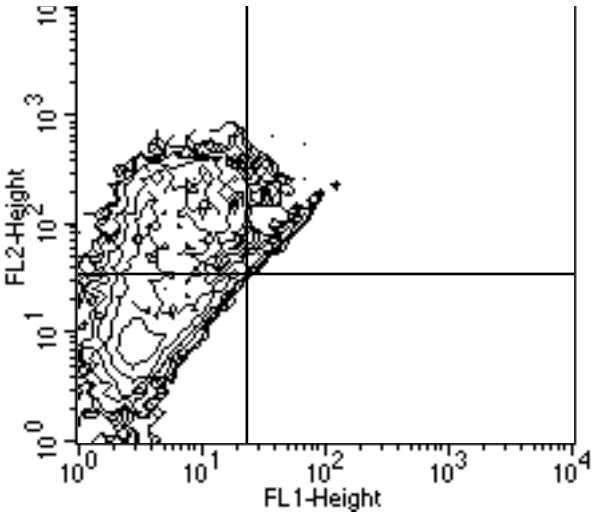
STORAGE CONDITIONS: ***Store at 2 - 5^{o}C. Do not freeze! Protect from light.***

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_3 (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 **250µg/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 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 permeabolized 10 minutes in a buffer containing **0.3% Saponin**. Subsequent reagent incubations and washes were done using this buffer. Five x 10^5 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/R-PE at a **1:50 dilution** (5 µg/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** $\log_{10}$ fluorescent units when compared to a Mouse IgG1/R-PE negative control (Catalog #278-050). Binding was blocked when cells were pre incubated 10 minutes with 20 µl of 0.5mg/ml anti-TNFα antibody (Catalog #398-020).



**** Research Use Only. Not for use in Diagnostic procedures.***

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