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Product Information

Monoclonal Anti-Tumor Necrosis Factor-α
clone 28401
produced in mouse, purified immunoglobulin

Catalog Number **T6817**

Product Description

Monoclonal Anti-Tumor Necrosis Factor-α (TNF-α) (mouse IgG1) is produced in mouse using purified recombinant human tumor necrosis factor-α, expressed in *E. coli,* as immunogen. The antibody is purified from ascites fluid using protein G affinity chromatography.

Monoclonal Anti-Tumor Necrosis Factor-α recognizes recombinant human tumor necrosis factor by various immunochemical techniques including immunoblotting, immunohistochemistry, neutralization, and capture ELISA. Using the antibody as a capture antibody in human TNF-α sandwich ELISAs, there is less than 0.05% cross-reactivity with recombinant human TNF-β, recombinant mouse TNF-α, recombinant rat TNF-α, recombinant porcine TNF-α, recombinant human TNF RI, recombinant human TNF RII, recombinant mouse TNF RI, and recombinant mouse TNF RII.

Tumor necrosis factor-α (TNF-α),[1-5] also called cachectin, is a member of the TNF superfamily of cytokines. TNF-α is expressed as a 26 kDa membrane bound protein and is then cleaved by TNF-α converting enzyme (TACE) to release the soluble 17 kDa monomer, which forms homotrimers in circulation. Human and mouse TNF-α share ~79% amino acid sequence identity. TNF-α and the related molecule TNF-β (LT-α) share close structural homology with 28% amino acid sequence identity and both activate the same TNF receptors, TNF RI and TNF RII.

Tumor necrosis factor-α plays roles in antitumor activity, immune modulation, inflammation, anorexia, cachexia, septic shock, viral replication, and hematopoiesis. It is expressed by a great variety of cells, with numerous inductive and suppressive agents.

Primarily, macrophages produce TNF-α in response to immunological challenges such as bacteria (lipopoly-saccharides), viruses, parasites, mitogens, and other cytokines. Neutrophils, activated lymphocytes, NK cells, LAK cells, astrocytes, endothelial cells, smooth muscle cells, and some transformed cells also produce TNF-α.

TNF-α is cytotoxic for many transformed cells (its namesake activity) but in normal diploid cells, it can stimulate proliferation (fibroblasts), differentiation (myeloid cells) or activation (neutrophils).[5] TNF-α also shows antiviral effects against both DNA and RNA viruses and induces production of several other cytokines.

Reagent

Supplied as ~500 μg of antiserum lyophilized from a 0.2 μm filtered solution in phosphate buffered saline containing 5% trehalose.

Preparation Instructions

To one vial of lyophilized powder, add 1 mL of sterile phosphate buffered saline containing 0.1% human serum albumin or bovine serum albumin to produce a 0.5 mg/mL stock solution of antibody.

Storage/Stability

Prior to reconstitution, store at –20 °C. Reconstituted product may be stored at 2-8 °C for up to one month. For prolonged storage, freeze in working aliquots at –20 °C. Avoid repeated freezing and thawing.

Procedure

Capture ELISA: the antibody can be used as the capture antibody in a human TGF-α ELISA in combination with biotinylated, human TGF-α affinity purified polyclonal detection antibody. Using plates coated with
100 μL/well of the capture antibody at 4 μg/mL, in combination with 100 μL/well of the detection antibody, an ELISA for sample volumes of 100 μL can be obtained. To arrive at the optimal dose range for this ELISA, a two-fold dilution series of the protein standard starting with 1 ng/mL is suggested.

Neutralization: the bioactivity was determined on mouse L929 cells[6] using the murine L929 cytotoxicity assay. To measure this activity, recombinant human TNF-α is incubated with various concentrations (0.0001-10 ng/mL) of the antibody in a 96 well plate. Following this preincubation period, mouse L929 cells are added.

The assay mixture is then incubated in a humidified CO_2 incubator. The exact concentration of antibody required to neutralize recombinant human TNF-α activity is dependent on the cytokine concentration, cell type, growth conditions, and the type of activity. The Neutralization $Dose_{50}$ (ND_{50}) for this antibody is defined as that concentration required to yield one-half maximal inhibition of the TNF-α activity on a responsive cell line, when TNF-α is present at a concentration just high enough to elicit a maximum response.

Product Profile

Capture ELISA: a working antibody concentration of 4 μg/mL is recommended to detect recombinant human TNF-α.

Neutralization: the antibody neutralizes the biological activity of recombinant human TNF-α in a mouse fibrosarcoma cell line (L929).

Immunoblotting: a working antibody concentration of 1-2 μg/mL detects human TNF-α at ~5 ng/lane under non-reducing and reducing conditions.

Immunohistochemistry: a minimum working antibody concentration of 25 μg/mL is recommended for detecting human TNF-α in fixed human peripheral blood leukocytes using the appropriate secondary reagents and chromogenic detection system.

Note: In order to obtain the best results in various techniques and preparations, we recommend determining the optimal working dilutions by titration.

Endotoxin: < 0.1 EU (endotoxin units) per 1 μg of the antibody as determined by the LAL method.

References

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4. Beutler, B., Cachectin/tumor necrosis factor and lymphotoxin, in Peptide Growth Factors and their Receptors II, Sporn, M., and Roberts, A., eds., Springer-Verlag, (New York, NY: 1991), pp. 39-70.
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TD,PHC 09/12-1