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ProductInformation

Anti-Tumor Necrosis Factor-a
produced in goat, affinity isolated antibody

Product Number **T0938**

Product Description

Anti-mouse Tumor Necrosis Factor-α (TNF-α) is developed in goat using recombinant mouse TNF-α expressed in *E. coli* as immunogen. The antibody is purified using mouse TNF-α affinity chromatography.

Anti-TNF-α will neutralize the biological activity of recombinant mouse TNF-α. The antibody may be used in immunoblotting, neutralization, ELISA capture, flow cytometry, and indirect immunofluorescence.

Tumor Necrosis Factor-α (TNF-α) is a protein secreted by lipopolysaccharide-stimulated macrophages, and causes tumor necrosis *in vivo* when injected into tumor-bearing mice.[1] Also known as cachectin, TNF-α is believed to mediate pathogenic shock and tissue injury associated with endotoxemia.[2] TNF-α exists as a multimer of two, three or five non-covalently linked units, but shows a single 17 kDa band with SDS-PAGE under non-reducing conditions.[3] TNF-α is closely related to the 25 kDa protein Tumor Necrosis Factor-β (lymphotoxin), sharing the same receptors and cellular actions.[4] TNF-α causes cytolysis or cytostasis of certain transformed cells,[5,6] being synergistic with interferon-γ in its cytotoxicity.[7] Although it has little effect on many cultured normal human cells,[6] TNF-α appears to be directly toxic to vascular endothelial cells.[8] Other actions of TNF-α include stimulating growth of human fibroblasts and other cell lines,[9] activating polymorphonuclear neutrophils[10] and osteoclasts,[11] and inducting of interleukin-1, prostaglandin E_2 and collagenase production.[12,13] The amino acid sequence of recombinant mouse TNF-α is 79% homologous with that of recombinant human TNF-α.[14]

Reagent

Lyophilized from phosphate buffered saline with 5% trehalose.

Storage/Stability

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.

Reconstitution

To one vial of lyophilized powder, add 1 mL of 0.2 µm filtered phosphate buffered saline to produce a 0.1 mg/mL stock solution of antibody. If aseptic technique is used, no further filtration should be needed for use in cell culture environments

Product Profile

This antibody is tested for its ability to neutralize the bioactivity of recombinnat mouse TNF-α in a cytotoxicity assay using murine L-929.[15] In this bioassay, recombinant mouse TNF-α was preincubated with various dilutions of the antibody for 1 hour at 37 °C in a 96-well plate. Confluent cultures of L929 cells are added to each well. The total volume of 150 µl, containing antibody, recombinant mouse TNF-α at 0.25 ng/ml, and actinomyocin D at 1 µg/mL is incubated for 24 hours at 37 °C in a 5% CO_2 humidified incubator. Cells are fixed with 5% formaldehyde and stained with crystal violet. The stain is dissolved with 100 µL 33% acetic acid and the absorbance at 540 nm is measured.

The ND_{50} of the antibody is defined as the concentration of antibody resulting in a one-half maximal inhibition of bioactivity of recombinant mouse TNF-α that is present at a concentration just high enough to elicit a maximum response.

For capture ELISAs, the anibody can be used as the capture antibody in a mouse TNF-α ELISA in combination with biotinylated, mouse TNF-α affinity purified

polyclonal detection antibody. Using plates coated with 100 µl/well of the capture antibody at 0.8 µg/mL, in combination with 100 µL/well of the detection antibody at 300 ng/mL, an ELISA for sample volumes of 100 µl can be obtained. Titrate each preparation of the recombinant protein for standard preparation to arrive at the most suitable dose range. For this ELISA, a two-fold dilution series starting at 4 ng/mL is suggested.

By immunoblotting, a working antibody concentration of 0.1- 0.2 µg/mL is recommended. The detection limit for recombinant mouse TNF-α is ~0.5 ng/lane under non-reducing and reducing conditions.

For flow cytometry, a working antibody concentration of 5-25 µg/mL (50-250 ng/10^6 cells) is recommended.

References

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