

Product Information

Anti-Tumor Necrosis Factor- α

antibody produced in rabbit, IgG fraction of antiserum

Catalog Number **T8300**

Synonym: Anti-TNF- α

Product Description

Anti-Tumor Necrosis Factor- α is produced in rabbit using purified recombinant human TNF- α as the immunogen. Whole antiserum is fractionated and then further purified by ion exchange chromatography to provide the IgG fraction of antiserum. This fraction is essentially free of other rabbit serum proteins.

Anti-TNF- α detects recombinant human TNF- α by dot blot. The product is negative versus recombinant human TNF- β , recombinant human IL-1 α , recombinant human IL-1 β , recombinant human IL-3, recombinant mouse, and human IL-6.

Anti-TNF- α may be used to study human TNF- α using various immunoassays including immunoblotting, dot blot, ELISA, and for selective neutralization of human TNF- α bioactivity in cell culture.

Tumor necrosis factor-alpha (TNF- α),^{1,2} also known as cachectin, is an inflammatory cytokine that exists primarily as a 51 kDa complex built up of 3 identical, non-covalently-linked polypeptide subunits (17 kDa, 157 amino acid residues, in human). TNF- α occurs as a secreted, soluble form and as a membrane-anchored form, both of which are biologically active. Membrane-bound TNF- α on activated human monocytes appears as a 26 kDa polypeptide translation product and includes an uncleaved 76 residue signal sequence. Although this leader peptide is removed co-translationally to yield the 17 kDa secreted form, it apparently persists in membrane-bound TNF. Murine cytotoxic T lymphocytes (CTL) appear to express a membrane protein of 50-60 kDa which is immunologically related to TNF- α . The naturally occurring form of TNF- α is glycosylated, but non-glycosylated recombinant TNF- α has comparable biological activity. Human and murine TNF- α show ~79% homology at the amino acid level.

Two types of receptors for TNF- α have been described and virtually all cell types studied show the presence of one or both of these receptor types. TNF- α shares 30% homology at the DNA level with TNF- β , both bind to the same receptor and have similar biological effects.

Many substances can induce the production of TNF, but bacterial cell wall products, such as lipopolysaccharides, are among the most potent inducers. TNF- α is produced by neutrophils, activated lymphocytes, macrophages, NK cells, LAK cells, astrocytes, endothelial cells, smooth muscle cells, and some transformed cells. TNF- α appears to be a major mediator of inflammatory responses and over-production results in the extensive tissue damage associated with endotoxemia and cachexia. In addition, high serum levels of TNF are associated with septic shock, rejection of renal transplants, parasitic infections, and various neoplastic diseases.

TNF- α influences the growth and function of both normal and neoplastic cells. On normal cells, TNF- α exhibits growth enhancing activities for fibroblasts, T cells, and B cells; functional activation of endothelial cells and neutrophils; and alterations of the growth and differentiation of myeloid cells.² On neoplastic cells, TNF- α exerts growth inhibitory activities which can be either cytostatic or cytotoxic. Cytostasis can reflect the ability of TNF- α to induce cellular differentiation.

Because these molecules play major roles in biological responses and can contribute to pathological states, an *in vitro* assay for their detection and quantification is desirable.³ A product which reacts specifically with TNF- α is a useful tool for the determination and quantification of the molecule in many *in vitro* systems and *in vivo* animal or human models.

Reagent

Supplied as a 0.2 μ m-filtered solution in 0.01 M phosphate buffered saline, pH 7.4.

Protein Concentration: minimum 5 mg/ml by extinction.
($E_{280}^{1\%} = 14.0$.)

Precautions and Disclaimer

This product is for R&D use only, not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Preparation Instructions

Dilute antibody in 0.2 μ m-filtered tissue culture medium containing 10% serum, or buffered saline containing 1% BSA, according to the planned application.

Storage/Stability

Store undiluted antibody at -20°C . The product should be stored frozen in working aliquots. Repeated freezing and thawing, or storage in "frost-free" freezers, is not recommended.

Product Profile

Bioactivity: an antibody concentration of 1-3 $\mu\text{g/ml}$ neutralizes 50% of the cytotoxicity of 1 unit of recombinant, human TNF- α using the mouse cell line A-9 (a derivative of the L connective tissue cell line).

Dot Blot: a minimum dilution of 1:2,000 was determined using 50 ng recombinant human TNF- α /dot on a nitrocellulose membrane.

References

1. Carswell, E. et al., *Proc. Natl. Acad. Sci. (USA)*, **72**, 3666 (1975).
2. Beutler, B., and Cerami, A., *Biochemistry*, **27**, 7575 (1988).
3. Baarsch, M. et al., *J. Immunol. Meth.*, **140**, 15 (1991).

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