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ProductInformation

Anti-Tumor Necrosis Factor a
Affinity Isolated Antibdoy
Antibody produced in goat

Product Number **T1816**

Product Description

Anti-porcine Tumor Necrosis Factor α (TNF-α) is produced in goat using as immunogen a purified recombinant porcine tumor necrosis factor α expressed in *E. coli*. TNF-α specific IgG was purified by porcine TNF-α affinity chromatography.

Anti-porcine Tumor Necrosis Factor α recognizes recombinant porcine tumor necrosis factor α by various immunochemical techniques including immunoblotting, neutralization, ELISA, and immunohistochemistry.

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. 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

The antibody is supplied as approximately 100 µg of antiserum lyophilized from a 0.2 µm filtered solution in phosphate buffered saline, pH 7.4, with 5% trehalose.

Preparation Instructions

To one vial of lyophilized powder, add 1 ml of sterile phosphate buffered saline to produce a 0.1 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. Avoid repeated freezing and thawing. Do not store in a frost-free freezer.

Procedure

To measure the ability of the antibody to neutralize the bioactivity of recombinant porcine TNF-α on porcine PK(15) cells, recombinant porcine TNF-α was incubated with various concentration of the antibody (0.01-30 µg/ml) for 1 hour at 37 °C. Following this preincubation period, the assay mixture was added to a

confluent culture of porcine PK(15) cells in a 96 well plate. The assay mixture in a total volume of 150 µl, containing antibody at the concentrations indicated, recombinant porcine TNF-α at 0.05 ng/ml, and actinomycin D at 1 µg/.ml was incubated for 24 hours at 37 °C in a 5% CO_2 humidified incubator. Following this incubation, the cells were fixed with 5% formaldehyde and stained with crystal violet. The stain was dissolved in 100 µl of 33% acetic acid and the absorbance at 540 nm (ref. 690 nm) was read on a plate reader.

Product Profile

Anti-porcine TNF-α has the ability to neutralize the biological activity of recombinant porcine TNF-α. The ND_{50} for this effect is 0.2-0.8 µg/ml in the presence of 0.05 ng/ml of recombinant porcine TNF-α and actinomycin D at 1 µg/ml using the porcine PK(15) cell line.

The exact concentration of antibody required to neutralize recombinant porcine 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.

For immunoblotting, a working antibody concentration of 0.1-0.2 µg/ml detects porcine TNF-α at approximately 5 ng/lane under non-reducing and reducing conditions.

For ELISAs, a working antibody concentration of 0.5-1.0 µg/ml is recommended to detect TNF-α. The detection limit for recombinant porcine TNF-α and recombinant human TNF-α is approx. 0.16 ng/well. The detection limit for recombinant mouse TNF-α, recombinant Rhesus Macaque TNF-α, recombinant Cotton Rat TNF-α, and recombinant rat TNF-α is approximately 0.6 ng/well.

By immunohistochemistry, a working antibody concentration of 15 µg/ml will detect TNF-α in activated porcine PBLs by either fluorescent or chromogenic detection systems.

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

Endotoxin: < 0.01 EU per 1 µg of the antibody as determined by the LAL method.

References

1. Ware, c., et al., Tumor necrosis factor-related ligands and receptors, in The Cytokine Handbook, 3rd Edition, Thomson, A.W., ed., Academic Press (San Diego, CA: 1998), pp. 549-592.
2. Aggarwal, B., and Reddy, S., Tumor necrosis factor (TNF), in Guidebook to Cytokines and Their Receptors, Nicola, N., ed., Oxford Press (New York, NY: 1994), pp. 103-104.
3. Callard, R., and Gearing, A., The Cytokine Facts Book, Academic Press (New York, NY: 1994).
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.
5. Beutler, B., and Cerami, A., The history, properties, and biological effects of cachectin. Biochemistry, **27**, 7575-7582 (1988).

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