Anti-equine TNF-α/TNFSF1A Antibody

ORDERING INFORMATION

Catalog Number: AF1814

Lot Number: UCO01

Size: 100 μg

Formulation: 0.2 μm filtered solution in PBS with 5% trehalose

Storage: -20° C

Reconstitution: sterile PBS

Specificity: equine TNF-α

Immunogen: *E. coli*-derived reqTNF-α

Ig Type: goat IgG

Applications: Neutralization of bioactivity
Western blot
ELISA
Immunocytochemistry

Preparation
Produced in goats immunized with purified, *E. coli*-derived, recombinant equine Tumor Necrosis Factor alpha (reqTNF-α). Equine TNF-α specific IgG was purified by equine TNF-α affinity chromatography.

Formulation
Lyophilized from a 0.2 μm filtered solution in phosphate-buffered saline (PBS) with 5% trehalose.

Endotoxin Level
< 0.1 EU per 1 μg of the antibody as determined by the LAL method.

Reconstitution
Reconstitute with sterile PBS. If 1 mL of PBS is used, the antibody concentration will be 0.1 mg/mL.

Storage
Lyophilized samples are stable for twelve months from date of receipt when stored at -20° C to -70° C. Upon reconstitution, the antibody can be stored at 2° - 8° C for 1 month without detectable loss of activity. Reconstituted antibody can also be aliquotted and stored frozen at -20° C to -70° C **in a manual defrost freezer** for six months without detectable loss of activity. **Avoid repeated freeze-thaw cycles.**

Specificity
This antibody has been selected for its ability to neutralize equine TNF-α bioactivity.

Neutralization of Equine TNF-α Bioactivity
The exact concentration of antibody required to neutralize equine TNF-α activity is dependent on the cytokine concentration, cell type, growth conditions and the type of activity studied. To provide a guideline, R&D Systems has determined the neutralization dose for this antibody under a specific set of conditions. The **Neutralization Dose$_{50}$ (ND$_{50}$)** for this antibody is defined as that concentration of antibody required to yield one-half maximal inhibition of the cytokine activity on a responsive cell line, when that cytokine is present at a concentration just high enough to elicit a maximum response.
The ND$_{50}$ for this lot of anti-equine TNF-α antibody was determined to be approximately 0.01 - 0.03 μg/mL in the presence of 1.0 ng/mL of reqTNF-α, using the mouse L929 cell line. The specific conditions are described in the figure legends.

Additional Applications
Direct ELISA - This antibody can be used at 0.5 - 1.0 μg/mL with the appropriate secondary reagents to detect equine and human TNF-α. The detection limit for reqTNF-α and rhTNF-α is approximately 1.5 ng/well.

Western blot - This antibody can be used at 0.1 - 0.2 μg/mL with the appropriate secondary reagents to detect equine and human TNF-α. The detection limit for reqTNF-α and rhTNF-α is approximately 1 ng/lane under non-reducing and reducing conditions. In this format, this antibody shows approximately 35% cross-reactivity with rmTNF-α and rrTNF-α.

Immunocytochemistry - This antibody will detect TNF-α in cells. The working dilution is 5 - 15 μg/mL. For chromogenic detection of labeling, use R&D Systems' Cell and Tissue Staining Kits (CTS Series).

Optimal dilutions should be determined by each laboratory for each application.

R&D Systems, Inc.
1-800-343-7475

Figure 1

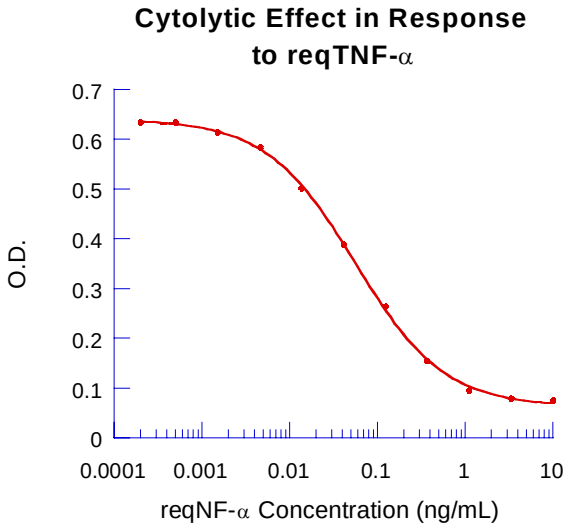

Figure 2

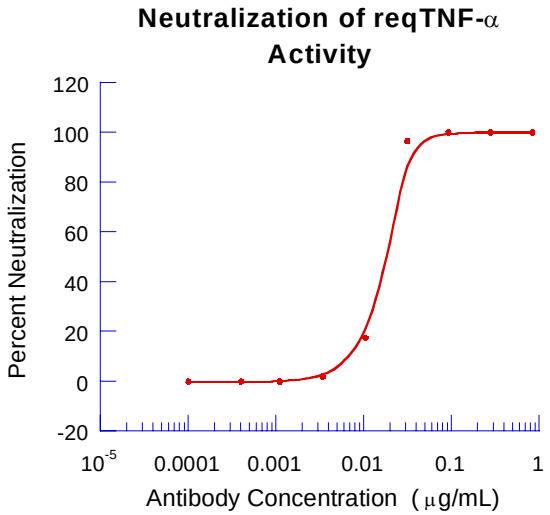

Figure 1
The biological activity of reqTNF-α was measured by its cytolytic effect on murine L-929 cells in the presence of actinomycin D (Matthews, N. *et al*., 1987, in *Lymphokines and Interferons, a practical approach*, M.J. Clemens, A.G. Morris and A.J.H. Gearing, eds., IRL Press, p. 221). The ED_{50} for this effect is typically 0.025 - 0.1 ng/mL.

Figure 2
To measure the ability of the antibody to neutralize the bioactivity of reqTNF-α on murine L-929 cells, reqTNF-α was incubated with various concentrations of antibody for 1 hour at 37° C. Following this preincubation period, the assay mixture was added to a confluent culture of murine L-929 cells in a 96 well microtiter plate. The assay mixture in a total volume of 150 μL, containing antibody at the concentrations indicated, reqTNF-α at 1 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 microtiter plate reader. The ND_{50} of the antibody is approximately 0.01 - 0.03 μg/mL.