Equine TNF-α Antibody

Antigen Affinity-purified Polyclonal Goat IgG
Catalog Number: AF1814

DESCRIPTION

Species Reactivity	Equine
Specificity	Detects equine TNF-alpha in direct ELISAs and Western blots. In Western blots, approximately 35% cross-reactivity with recombinant mouse TNF-alpha and recombinant rat TNF-alpha is observed.
Source	Polyclonal Goat IgG
Purification	Antigen Affinity-purified
Immunogen	*E. coli*-derived recombinant equine TNF-alpha Leu78-Leu234 Accession # NP_001075288
Endotoxin Level	<0.10 EU per 1 µg of the antibody by the LAL method.
Formulation	Lyophilized from a 0.2 µm filtered solution in PBS with Trehalose. See Certificate of Analysis for details. *Small pack size (-SP) is supplied either lyophilized or as a 0.2 µm filtered solution in PBS.

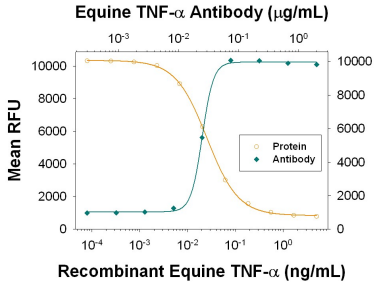
APPLICATIONS

Please Note: *Optimal dilutions should be determined by each laboratory for each application. General Protocols are available in the Technical Information section on our website.*

	Recommended Concentration	Sample
Western Blot	0.1 µg/mL	Recombinant Equine TNF-α (Catalog # 1814-ET)
Immunocytochemistry	5-15 µg/mL	See Below
Neutralization	Measured by its ability to neutralize TNF-α-induced cytotoxicity in the L-929 mouse fibroblast cell line. The Neutralization Dose (ND_{50}) is typically 0.01-0.03 µg/mL in the presence of 1 ng/mL Recombinant Equine TNF-α and 1 µg/mL actinomycin D.	

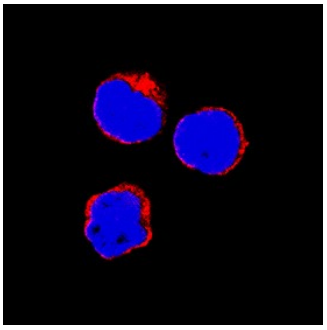
DATA

Neutralization



Cytotoxicity Induced by TNF-α and Neutralization by Equine TNF-α Antibody. Recombinant Equine TNF-α (Catalog # 1814-ET) induces cytotoxicity in the the L-929 mouse fibroblast cell line in a dose-dependent manner (orange line), as measured by Resazurin (Catalog # AR002). Cytotoxicity elicited by Recombinant Equine TNF-α (1 ng/mL) is neutralized (green line) by increasing concentrations of Goat Anti-Equine TNF-α Antigen Affinity-purified Polyclonal Antibody (Catalog # AF1814). The ND_{50} is typically 0.01-0.03 µg/mL in the presence of the metabolic inhibitor actinomycin D (1 µg/mL).

Immunocytochemistry



TNF-α in Equine PBMCs. TNF-α was detected in immersion fixed equine peripheral blood mononuclear cells (PBMCs) using Goat Anti-Equine TNF-α Antigen Affinity-purified Polyclonal Antibody (Catalog # AF1814) at 15 µg/mL for 3 hours at room temperature. Cells were stained using the NorthernLights™ 557-conjugated Anti-Goat IgG Secondary Antibody (red; Catalog # NL001) and counterstained with DAPI (blue). Specific staining was localized to cytoplasm. View our protocol for Fluorescent ICC Staining of Non-adherent Cells.

PREPARATION AND STORAGE

Reconstitution	Reconstitute at 0.2 mg/mL in sterile PBS.
Shipping	The product is shipped at ambient temperature. Upon receipt, store it immediately at the temperature recommended below. *Small pack size (-SP) is shipped with polar packs. Upon receipt, store it immediately at -20 to -70 °C
Stability & Storage	**Use a manual defrost freezer and avoid repeated freeze-thaw cycles.** ● 12 months from date of receipt, -20 to -70 °C as supplied. ● 1 month, 2 to 8 °C under sterile conditions after reconstitution. ● 6 months, -20 to -70 °C under sterile conditions after reconstitution.

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BACKGROUND

Tumor necrosis factor alpha (TNF-α) also known as cachectin and TNFSF2, is the prototypic ligand of the TNF superfamily. It is a pleiotropic molecule that plays a central role in inflammation, apoptosis, and immune system development. TNF-α is produced by a wide variety of immune and epithelial cell types (1, 2). Equine TNF-α consisits of a 35 amino acid (aa) cytoplasmic domain, a 21 aa transmembrane segment, and a 178 aa extracellular domain (ECD) (3). Within the ECD, equine TNF-α shares 69%-88% aa sequence identity with bovine, canine, cotton rat, feline, human, mouse, porcine, rat, and rhesus TNF-α. The 26 kDa type 2 transmembrane protein is assembled intracellularly to form a noncovalently linked homotrimer (4). Ligation of this complex induces reverse signaling that promotes lymphocyte costimulation but diminishes monocyte responsiveness (5). Cleavage of membrane bound TNF-α by TACE/ADAM17 releases a 55 kDa soluble trimeric form of TNF-α (6, 7). TNF-α trimers bind the ubiquitous TNF RI and the hematopoietic cell-restricted TNF RII, both of which are also expressed as homotrimers (1, 8). TNF-α regulates lymphoid tissue development through control of apoptosis (2). It also promotes inflammatory responses by inducing the activation of vascular endothelial cells and macrophages (2). TNF-α is a key cytokine in the development of several inflammatory disorders (9). It contributes to the development of type 2 diabetes through its effects on insulin resistance and fatty acid metabolism (10, 11).

References:

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