Canine TNF-α Antibody

Antigen Affinity-purified Polyclonal Goat IgG
Catalog Number: AF1507

DESCRIPTION

Species Reactivity	Canine
Specificity	Detects canine TNF-α direct ELISAs and Western blots. In direct ELISAs, approximately 70% cross-reactivity with recombinant human TNF-α, 50% cross-reactivity with recombinant rhesus macaque TNF-α, 35% cross-reactivity with recombinant equine TNF-α, recombinant cotton rat TNF-α and recombinant rat TNF-α, 25% cross-reactivity with recombinant mouse TNF-α, and 10% cross-reactivity with recombinant porcine TNF-α is observed.
Source	Polyclonal Goat IgG
Purification	Antigen Affinity-purified
Immunogen	*E. coli*-derived recombinant canine TNF-α Val77-Leu233 Accession # P51742
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 as a 0.2 µm filtered solution in PBS.

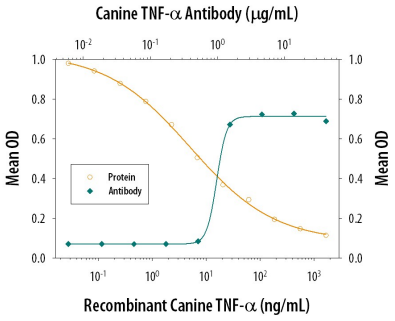
APPLICATIONS

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 Canine TNF-α (Catalog # 1507-CT)
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. Matthews, N. and M.L. Neale (1987) in Lymphokines and Interferons, A Practical Approach. Clemens, M.J. *et al.* (eds): IRL Press. 221. The Neutralization Dose (ND_{50}) is typically 0.4-1.6 µg/mL in the presence of 100 ng/mL Recombinant Canine TNF-α and 1 µg/mL actinomycin D.	

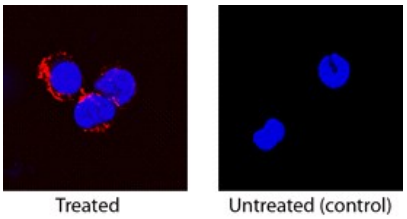
DATA

Neutralization



Cytotoxicity Induced by TNF-α and Neutralization by Canine TNF-α Antibody. Recombinant Canine TNF-α (Catalog # 1507-CT) induces cytotoxicity in the the L-929 mouse fibroblast cell line in a dose-dependent manner (orange line), as measured by crystal violet staining. Cytotoxicity elicited by Recombinant Canine TNF-α (100 ng/mL) is neutralized (green line) by increasing concentrations of Goat Anti-Canine TNF-α Antigen Affinity-purified Polyclonal Antibody (Catalog # AF1507). The ND_{50} is typically 0.4-1.6 µg/mL in the presence of the metabolic inhibitor actinomycin D (1 µg/mL).

Immunocytochemistry



TNF-α in Canine PBMCs. TNF-α was detected in immersion fixed canine peripheral blood mononuclear cells (PBMCs) stimulated with PMA and calcium ionomycin using Goat Anti-Canine TNF-α Antigen Affinity-purified Polyclonal Antibody (Catalog # AF1507) 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). Canine TNF-α consisits of a 35 amino acid (aa) cytoplasmic domain, a 21 aa transmembrane segment, and a 177 aa extracellular domain (ECD) (3). Within the ECD, canine TNF-α shares 84-94% aa sequence identity with equine, feline, human, porcine, and rhesus and 69-77% with bovine, cotton rat, mouse, and rat with 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 co-stimulation 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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