

DESCRIPTION

Species Reactivity	Canine
Specificity	Detects canine TNF- α direct ELISAs and Western blots.
Source	Polyclonal Goat IgG
Purification	Antigen Affinity-purified
Immunogen	<i>E. coli</i> -derived recombinant canine TNF- α Val77-Leu233 Accession # P51742
Endotoxin Level	<0.10 EU per 1 μ g of the antibody by the LAL method.
Formulation	Lyophilized from a 0.2 μ m 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 μ m filtered solution in PBS.

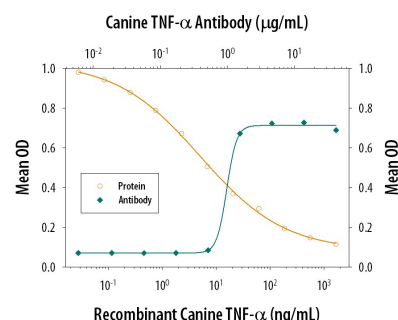
APPLICATIONS

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 μ g/mL	Recombinant Canine TNF- α (Catalog # 1507-CT)
Immunocytochemistry	5-15 μ g/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 <i>Lymphokines and Interferons, A Practical Approach</i> . Clemens, M.J. <i>et al.</i> (eds): IRL Press. 221. The Neutralization Dose (ND ₅₀) is typically 0.4-1.6 μ g/mL in the presence of 100 ng/mL Recombinant Canine TNF- α and 1 μ g/mL actinomycin D.	

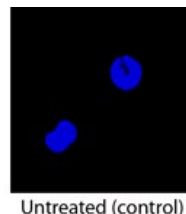
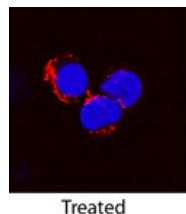
DATA

Neutralization



Cytotoxicity Induced by TNF- α and Neutralization by Canine TNF- α Antibody. Recombinant Canine TNF- α (Catalog # 1507-CT) induces cytotoxicity in the 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 ND₅₀ is typically 0.4-1.6 μ g/mL in the presence of the metabolic inhibitor actinomycin D (1 μ g/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 μ g/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. <ul style="list-style-type: none"> 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.

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 consists 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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