

DESCRIPTION

Species Reactivity	Canine
Specificity	Detects canine TNF- α in direct ELISAs. In direct ELISAs, 25-100% cross-reactivity with recombinant human TNF- α , recombinant porcine TNF- α , and recombinant rhesus macaque TNF- α is observed.
Source	Monoclonal Mouse IgG ₁ Clone # 236812
Purification	Protein A or G purified from hybridoma culture supernatant
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
Immunocytochemistry	8-25 μ g/mL	See Below
Canine TNF-α Sandwich Immunoassay		Reagent
ELISA Capture	2-8 μ g/mL	Canine TNF- α Antibody (Catalog # MAB1507)
ELISA Detection	0.1-0.4 μ g/mL	Canine TNF- α Biotinylated Antibody (Catalog # BAF1507)
Standard		Recombinant Canine TNF- α (Catalog # 1507-CT)
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 1.25-5.0 μ g/mL in the presence of 10 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). Cytotoxicity elicited by Recombinant Canine TNF- α (10 ng/mL) is neutralized (green line) by increasing concentrations of Mouse Anti-Canine TNF- α Monoclonal Antibody (Catalog # MAB1507). The ND₅₀ is typically 1.25-5.0 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) untreated or treated with calcium ionomycin and PMA using Mouse Anti-Canine TNF- α Monoclonal Antibody (Catalog # MAB1507) at 15 μ g/mL for 3 hours at room temperature. Cells were stained using the NorthernLights™ 557-conjugated Anti-Mouse IgG Secondary Antibody (red; Catalog # NL007) and counterstained with DAPI (blue). Specific staining was localized to cell secretion. View our protocol for [Fluorescent ICC Staining of Non-adherent Cells](#).

PREPARATION AND STORAGE

Reconstitution	Reconstitute at 0.5 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, 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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