

DESCRIPTION

Species Reactivity	Rat
Specificity	Detects rat TNF- α in direct ELISAs and Western blots. In Western blots, approximately 25% cross-reactivity with recombinant porcine TNF- α is observed and less than 1% cross-reactivity with recombinant human (rh) TNF- β , rhTNF sRI, and rhTNF sRII is observed. In direct ELISAs, approximately 40% cross-reactivity with recombinant canine TNF- α , recombinant feline TNF- α , and recombinant rhesus macaque TNF- α is observed. Neutralizes the biological activity of rat TNF- α . This antibody will also neutralize the biological activity of murine TNF- α , but weak neutralization of rhTNF- α and no neutralization of rhTNF- β was observed.
Source	Polyclonal Goat IgG
Purification	Antigen Affinity-purified
Immunogen	<i>E. coli</i> -derived recombinant rat TNF- α Leu80-Leu235 Accession # P16599
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 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 Rat TNF- α (Catalog # 510-RT)
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 0.15-0.75 μ g/mL in the presence of 0.25 ng/mL Recombinant Rat TNF- α and 1 μ g/mL actinomycin D.	

DATA

Neutralization

Cytotoxicity Induced by TNF- α and Neutralization by Rat TNF- α Antibody. Recombinant Rat TNF- α (Catalog # 510-RT) induces cytotoxicity in the L-929 mouse fibroblast cell line in a dose-dependent manner (orange line), as measured by Resazurin. Cytotoxicity elicited by Recombinant Rat TNF- α (0.25 ng/mL) is neutralized (green line) by increasing concentrations of Goat Anti-Rat TNF- α Antigen Affinity-purified Polyclonal Antibody (Catalog # AF-510-NA). The ND₅₀ is typically 0.15-0.75 μ g/mL in the presence of the metabolic inhibitor actinomycin D (1 μ g/mL).

Immunocytochemistry

TNF- α in Rat Splenocytes. TNF- α was detected in immersion fixed rat splenocytes using Goat Anti-Rat TNF- α Antigen Affinity-purified Polyclonal Antibody (Catalog # AF-510-NA) 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 cytoplasmic. 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). Rat TNF- α consists of a 35 amino acid (aa) cytoplasmic domain, a 21 aa transmembrane segment, and a 179 aa extracellular domain (ECD) (3). Within the ECD, rat TNF- α shares 94% aa sequence identity with mouse and 69% - 76% with bovine, canine, cotton rat, equine, feline, human, porcine, 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 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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