Mouse TNF-α Antibody

Antigen Affinity-purified Polyclonal Goat IgG
Catalog Number: AF-410-NA

DESCRIPTION

Species Reactivity	Mouse
Specificity	Detects mouse TNF-α in ELISAs and Western blots. In sandwich immunoassays, approximately 50% cross-reactivity with recombinant rat TNF-α is observed and less than 1% cross-reactivity with recombinant human TNF-α is observed.
Source	Polyclonal Goat IgG
Purification	Antigen Affinity-purified
Immunogen	*E. coli*-derived recombinant mouse TNF-α (R&D Systems, Catalog # 410-MT) Leu80-Leu235 Accession # P06804
Endotoxin Level	<0.1 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.

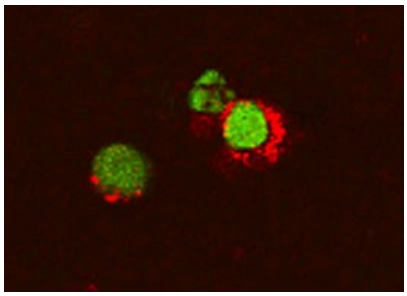
APPLICATIONS

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 Mouse TNF-α (Catalog # 410-MT)
Immunocytochemistry	5-15 µg/mL	See Below
Intracellular Staining by Flow Cytometry	2.5 µg/10^6 cells	RAW 264.7 mouse monocyte/macrophage cell line treated with LPS, fixed with paraformaldehyde, and permeabilized with saponin
Mouse TNF-α Sandwich Immunoassay		**Reagent**
ELISA Capture	0.2-0.8 µg/mL	Mouse TNF-α Antibody (Catalog # AF-410-NA)
ELISA Detection	0.1-0.4 µg/mL	Mouse TNF-α Biotinylated Antibody (Catalog # BAF410)
Standard		Recombinant Mouse TNF-α (Catalog # 410-MT)
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 0.1-0.4 µg/mL in the presence of 0.25 ng/mL Recombinant Mouse TNF-α and 1 µg/mL actinomycin D.	

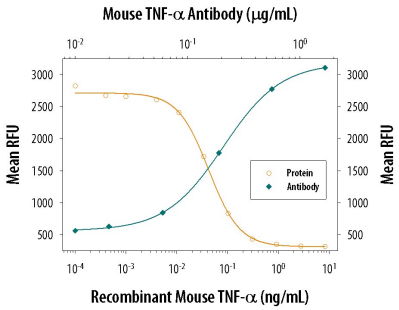
DATA

Immunocytochemistry



TNF-α in Mouse T Cells. TNF-α was detected in immersion fixed activated mouse T Cells using 15 µg/mL Mouse TNF-α Antigen Affinity-purified Polyclonal Antibody (Catalog # AF-410-NA) for 3 hours at room temperature. Cells were stained (red) and counterstained (green). View our protocol for Fluorescent ICC Staining of Non-adherent Cells.

Neutralization



Cytotoxicity Induced by TNF-α and Neutralization by Mouse TNF-α Antibody. Recombinant Mouse TNF-α (Catalog # 410-MT) induces cytotoxicity in the the L-929 mouse fibroblast cell line in a dose-dependent manner (orange line). Cytotoxicity elicited by Recombinant Mouse TNF-α (0.25 ng/mL) is neutralized (green line) by increasing concentrations of Mouse TNF-α Antigen Affinity-purified Polyclonal Antibody (Catalog # AF-410-NA). The ND_{50} is typically 0.1-0.4 µg/mL in the presence of the metabolic inhibitor actinomycin D (1 µg/mL).

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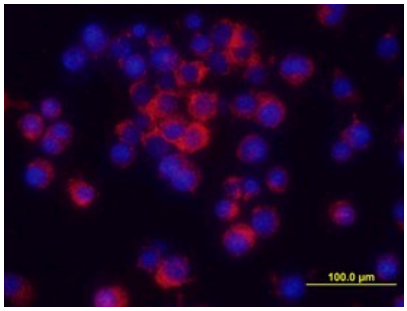

TNF-α in RAW 264.7 Mouse Cell Line. TNF-α was detected in immersion fixed RAW 264.7 mouse monocyte/macrophage cell line treated with LPS using Mouse TNF-α Antigen Affinity-purified Polyclonal Antibody (Catalog # AF-410-NA) at 10 µg/mL for 3 hours at room temperature. Cells were stained using the NorthernLights™ 557-conjugated Anti-Goat IgG Secondary Antibody (red; Catalog # NL001) and counterstained with DAPI (blue). View our protocol for Fluorescent ICC Staining of Cells on Coverslips.

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.
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 from date of receipt, 2 to 8 °C, reconstituted. ● 6 months from date of receipt, -20 to -70 °C, reconstituted.

BACKGROUND

Tumor necrosis factor alpha (TNF-α, also known as cachectin and TNFSF1A, 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. Mouse TNF-α consisits of a 35 amino acid (aa) cytoplasmic domain, a 21 aa transmembrane segment, and a 179 aa extracellular domain (ECD). Within the ECD, mouse TNF-α shares 94% aa sequence identity with rat and 70% - 77% with bovine, canine, cotton rat, equine, feline, human, porcine, rat, and rhesus TNF-α. The 26 kDa type 2 transmembrane protein is assembled intracellularly to form a noncovalently linked homotrimer. Ligation of this complex induces reverse signaling that promotes lymphocyte costimulation but diminishes monocyte responsiveness. Cleavage of membrane bound TNF-α by TACE/ADAM17 releases a 55 kDa soluble trimeric form of TNF-α. TNF-α trimers bind the ubiquitous TNF RI and the hematopoietic cell-restricted TNF RII, both of which are also expressed as homotrimers. TNF-α regulates lymphoid tissue development through control of apoptosis. It also promotes inflammatory responses by inducing the activation of vascular endothelial cells and macrophages. TNF-α is a key cytokine in the development of several inflammatory disorders. It contributes to the development of type 2 diabetes through its effects on insulin resistance and fatty acid metabolism.