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
<0.10 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 either lyophilized or 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 Mouse TNF-α (Catalog # 410-MT)

Immunocytochemistry
5-15 μg/mL
See Below

Intracellular Staining by Flow Cytometry
2.5 μg/10⁶ cells
RAW 264.7 mouse monocyte/macrophage cell line treated with LPS, fixed with paraformaldehyde, and permeabilized with saponin

Mouse TNF-α Sandwich Immunoassay
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)

Recombinant Mouse TNF-α aa 80-235 (Catalog # 410-MT)

CyTOF-ready
Ready to be labeled using established conjugation methods. No BSA or other carrier proteins that could interfere with conjugation.

Neutralization
Measured by its ability to neutralize TNF-α-induced cytotoxicity in the L929 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 (ND₅₀) 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 L929 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 ND₅₀ is typically 0.1-0.4 μg/mL in the presence of the metabolic inhibitor actinomycin D (1 μg/mL).
Immunocytochemistry

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.
*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.

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. Mouse TNF-α consists 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.