

## DESCRIPTION

<b>Species Reactivity</b>	Mouse
<b>Specificity</b>	Detects mouse TNF- $\alpha$ in ELISAs and Western blots. In sandwich immunoassays, approximately 50% cross-reactivity with recombinant rat TNF- $\alpha$ is observed and less than 1% cross-reactivity with recombinant human TNF- $\alpha$ is observed.
<b>Source</b>	Polyclonal Goat IgG
<b>Purification</b>	Antigen Affinity-purified
<b>Immunogen</b>	<i>E. coli</i> -derived recombinant mouse TNF- $\alpha$ (R&D Systems, Catalog # 410-MT) Leu80-Leu235 Accession # P06804
<b>Endotoxin Level</b>	<0.10 EU per 1 $\mu$ g of the antibody by the LAL method.
<b>Formulation</b>	Lyophilized from a 0.2 $\mu$ m filtered solution in PBS with Trehalose. See Certificate of Analysis for details. *Small pack size (-SP) is supplied as a 0.2 $\mu$ m filtered solution in PBS.

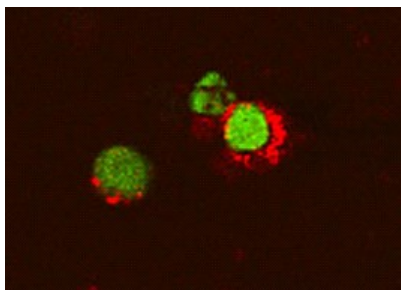
## APPLICATIONS

**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
<b>Western Blot</b>	0.1 $\mu$ g/mL	Recombinant Mouse TNF- $\alpha$ (Catalog # 410-MT)
<b>Immunocytochemistry</b>	5-15 $\mu$ g/mL	See Below
<b>Intracellular Staining by Flow Cytometry</b>	2.5 $\mu$ g/10 <sup>6</sup> cells	RAW 264.7 mouse monocyte/macrophage cell line treated with LPS, fixed with paraformaldehyde, and permeabilized with saponin
<b>Mouse TNF-<math>\alpha</math> Sandwich Immunoassay</b>		<b>Reagent</b>
<b>ELISA Capture</b>	0.2-0.8 $\mu$ g/mL	Mouse TNF- $\alpha$ Antibody (Catalog # AF-410-NA)
<b>ELISA Detection Standard</b>	0.1-0.4 $\mu$ g/mL	Mouse TNF- $\alpha$ Biotinylated Antibody (Catalog # BAF410) Recombinant Mouse TNF- $\alpha$ aa 80-235 (Catalog # 410-MT)
<b>CyTOF-ready</b>	Ready to be labeled using established conjugation methods. No BSA or other carrier proteins that could interfere with conjugation.	
<b>Neutralization</b>	Measured by its ability to neutralize TNF- $\alpha$ -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 <sub>50</sub> ) is typically 0.1-0.4 $\mu$ g/mL in the presence of 0.25 ng/mL Recombinant Mouse TNF- $\alpha$ and 1 $\mu$ g/mL actinomycin D.	

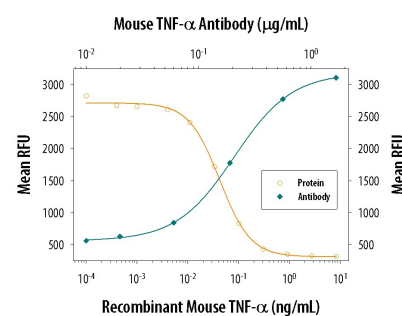
## DATA

### Immunocytochemistry



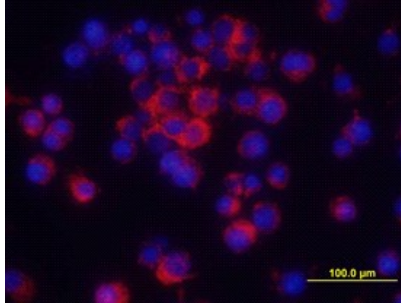
**TNF- $\alpha$  in Mouse T Cells.** TNF- $\alpha$  was detected in immersion fixed activated mouse T Cells using 15  $\mu$ g/mL Mouse TNF- $\alpha$  Antigen Affinity-purified Polyclonal Antibody (Catalog # AF-410-NA) for 3 hours at room temperature. Cells were stained (red) and counterstained (green).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

### Neutralization



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

**Immunocytochemistry**



**TNF- $\alpha$  in RAW 264.7 Mouse Cell Line.** TNF- $\alpha$  was detected in immersion fixed RAW 264.7 mouse monocyte/macrophage cell line treated with LPS using Mouse TNF- $\alpha$  Antigen Affinity-purified Polyclonal Antibody (Catalog # AF-410-NA) at 10  $\mu$ 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). View our protocol for [Fluorescent ICC Staining of Cells on Coverslips](#).

**PREPARATION AND STORAGE**

<b>Reconstitution</b>	Reconstitute at 0.2 mg/mL in sterile PBS.
<b>Shipping</b>	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
<b>Stability &amp; Storage</b>	<b>Use a manual defrost freezer and avoid repeated freeze-thaw cycles.</b> <ul style="list-style-type: none"> <li>● 12 months from date of receipt, -20 to -70 °C as supplied.</li> <li>● 1 month, 2 to 8 °C under sterile conditions after reconstitution.</li> <li>● 6 months, -20 to -70 °C under sterile conditions after reconstitution.</li> </ul>

**BACKGROUND**

Tumor necrosis factor alpha (TNF- $\alpha$ , 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- $\alpha$  is produced by a wide variety of immune and epithelial cell types. Mouse TNF- $\alpha$  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- $\alpha$  shares 94% aa sequence identity with rat and 70% - 77% with bovine, canine, cotton rat, equine, feline, human, porcine, rat, and rhesus TNF- $\alpha$ . 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- $\alpha$  by TACE/ADAM17 releases a 55 kDa soluble trimeric form of TNF- $\alpha$ . TNF- $\alpha$  trimers bind the ubiquitous TNF RI and the hematopoietic cell-restricted TNF RII, both of which are also expressed as homotrimers. TNF- $\alpha$  regulates lymphoid tissue development through control of apoptosis. It also promotes inflammatory responses by inducing the activation of vascular endothelial cells and macrophages. TNF- $\alpha$  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.