

DESCRIPTION

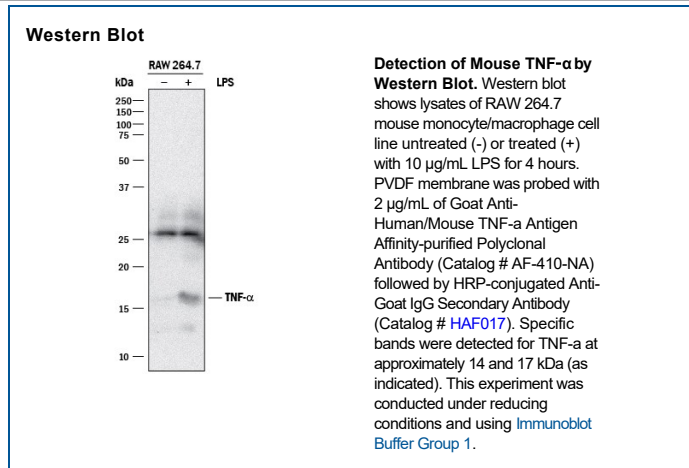
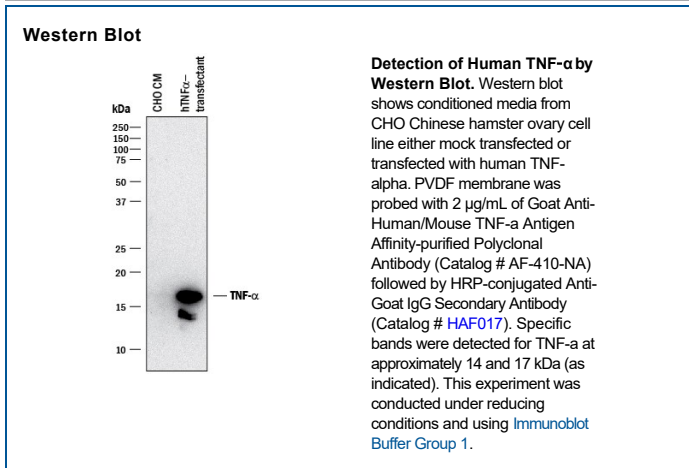
Species Reactivity	Human/Mouse
Specificity	Detects mouse TNF- α in ELISAs. Detects human and mouse TNF- α in Western blots. In sandwich immunoassays, approximately 50% cross-reactivity with recombinant rat TNF- α is observed.
Source	Polyclonal Goat IgG
Purification	Antigen Affinity-purified
Immunogen	<i>E. coli</i> -derived recombinant mouse TNF- α Leu80-Leu235 Accession # P06804
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

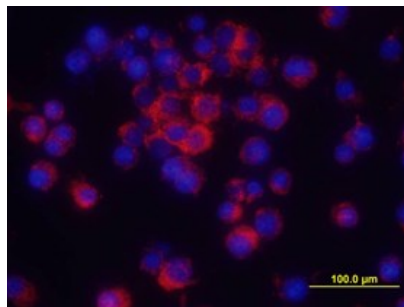
Please Note: Optimal dilutions should be determined by each laboratory for each application. [General Protocols](#) are available in the Technical Information section on our website.

	Recommended Concentration	Sample
Western Blot	2 μ g/mL	See Below
Immunocytochemistry	5-15 μ g/mL	See Below
Immunohistochemistry	3-15 μ g/mL	Immersion fixed paraffin-embedded sections of Mouse Spleen and Human Spleen.
Intracellular Staining by Flow Cytometry	2.5 μ g/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 μ g/mL	Human/Mouse TNF- α Antibody (Catalog # AF-410-NA)
ELISA Detection	0.1-0.4 μ g/mL	Mouse TNF- α Biotinylated Antibody (Catalog # BAF410)
Standard		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 L-929 mouse fibroblast cell line. Matthews, N. and M.L. Neale (1987) in Lymphokines and Interferons, A Practical Approach. Clemens, M.J. <i>et al.</i> (eds): IRL Press. 221. The Neutralization Dose (ND ₅₀) is typically 1.5-10 ng/mL in the presence of 0.1 ng/mL Recombinant Mouse TNF- α and actinomycin D.	

DATA

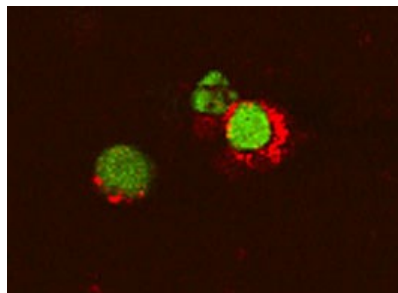


Immunocytochemistry



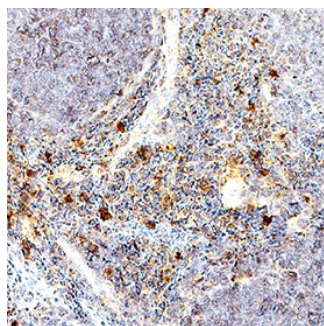
TNF-α in RAW 264.7 Mouse Cell Line. TNF-α was detected in immersion fixed RAW 264.7 mouse monocyte/ macrophage cell line treated with LPS using Human/Mouse TNF-α Antigen Affinity-purified Polyclonal Antibody (Catalog # AF-410-NA) at 10 μg/mL for 3 hours at room temperature. Cells were stained using the 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](#).

Immunocytochemistry



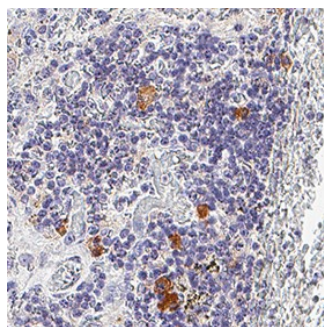
TNF-α in Mouse T Cells. TNF-α was detected in immersion fixed activated mouse T Cells using 15 μg/mL Human/Mouse TNF-α Antigen Affinity-purified Polyclonal Antibody (Catalog # AF-410-NA) for 3 hours at room temperature. Cells were stained (red) and counterstained (green). View our protocol for [Fluorescent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

Immunohistochemistry



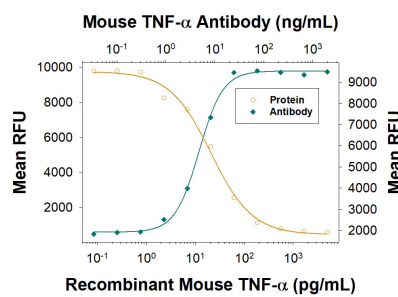
TNF-α in Mouse Spleen Tissue. TNF-α was detected in immersion fixed paraffin-embedded sections of mouse spleen tissue using Goat Anti-Human/Mouse TNF-α Antigen Affinity-purified Polyclonal Antibody (Catalog # AF-410-NA) at 3 μg/mL for 1 hour at room temperature followed by incubation with the Anti-Goat IgG VisUCyte™ HRP Polymer Antibody (Catalog # VC004). Tissue was stained using DAB (brown) and counterstained with hematoxylin (blue). Specific staining was localized to lymphocytes. Staining was performed using our [IHC Staining with VisUCyte HRP Polymer Detection Reagents](#).

Immunohistochemistry



Detection of TNF-α in Human Spleen. TNF-α was detected in immersion fixed paraffin-embedded sections of Human Spleen using Goat Anti-Human/Mouse TNF-α Antigen Affinity-purified Polyclonal Antibody (Catalog # AF-410-NA) at 5 μg/mL for 1 hour at room temperature followed by incubation with the Anti-Goat IgG VisUCyte™ HRP Polymer Antibody (Catalog # VC004). Before incubation with the primary antibody, tissue was subjected to heat-induced epitope retrieval using VisUCyte Antigen Retrieval Reagent-Basic (Catalog # VCTS021). Tissue was stained using DAB (brown) and counterstained with hematoxylin (blue). Specific staining was localized to cytoplasm in lymphocytes. View our protocol for [IHC Staining with VisUCyte HRP Polymer Detection Reagents](#).

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 (0.1 ng/mL) is neutralized (green line) by increasing concentrations of Human/Mouse TNF-α Antigen Affinity-purified Polyclonal Antibody (Catalog # AF-410-NA). The ND₅₀ is typically 1.5-10 ng/mL in the presence of the metabolic inhibitor actinomycin D.

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	<p>Use a manual defrost freezer and avoid repeated freeze-thaw cycles.</p> <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-alpha, TNF- α , TNFA), 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, immune system development, apoptosis, and lipid metabolism. TNF-alpha is produced by several lymphoid cells as well as by astrocytes, endothelial cells, and smooth muscle cells. 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, and rhesus TNF-alpha. TNF-alpha is produced by a wide variety of immune, epithelial, endothelial, and tumor cells. TNF-alpha is assembled intracellularly to form a noncovalently linked homotrimer which is expressed on the cell surface. Cell surface TNF-alpha can induce the lysis of neighboring tumor cells and virus infected cells, and it can generate its own downstream cell signaling following ligation by soluble TNFR I. Shedding of membrane bound TNF-alpha by TACE/ADAM17 releases the bioactive cytokine, a 55 kDa molecular weight soluble trimer of the TNF-alpha extracellular domain. TNF-alpha binds the ubiquitous 55-60 kDa TNF RI and the hematopoietic cell-restricted 80 kDa TNF RII, both of which are also expressed as homotrimers present on virtually all cell types. Both type I and type II receptors bind TNF-alpha with comparable affinity, although only TNF RI contains a cytoplasmic death domain which triggers the activation of apoptosis. Soluble forms of both types of receptors are released and can neutralize the biological activity of TNF-alpha.