Human TNF-α Antibody

Monoclonal Mouse IgG_1 Clone # 28401

Catalog Number: MAB610

DESCRIPTION

Species Reactivity	Human
Specificity	Detects human TNF-α in ELISAs and Western blots. In sandwich ELISAs, less than 0.05% cross-reactivity with recombinant human TNF-β, recombinant mouse TNF-α, recombinant rat TNF-α, and recombinant porcine TNF-α is observed.
Source	Monoclonal Mouse IgG_1 Clone # 28401
Purification	Protein A or G purified from hybridoma culture supernatant
Immunogen	*E. coli*-derived recombinant human TNF-α Gly57-Leu233 (predicted) Accession # P01375
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.

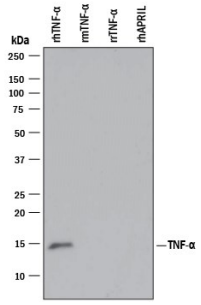
APPLICATIONS

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	1 µg/mL	See Below
Immunocytochemistry	8-25 µg/mL	See Below
Human TNF-α Sandwich Immunoassay		**Reagent**
ELISA Capture	2-8 µg/mL	Human TNF-α Antibody (Catalog # MAB610)
ELISA Detection	0.1-0.4 µg/mL	Human TNF-α Biotinylated Antibody (Catalog # BAF210)
Standard		Recombinant Human TNF-α (Catalog # 210-TA)
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 0.01-0.04 µg/mL in the presence of actinomycin D and 0.25 ng/mL Recombinant Human TNF-α.	

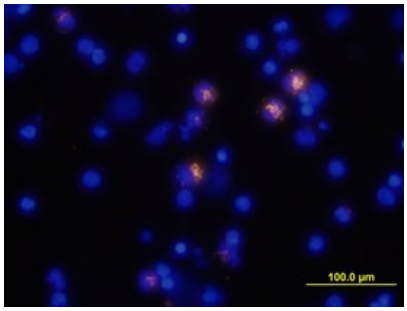
DATA

Western Blot



Detection of Recombinant Human TNF-α by Western Blot. Western blot shows 25 ng of Recombinant Human TNF-α (Catalog # 210-TA), Recombinant Mouse TNF-α aa 80-235 (Catalog # 410-MT), Recombinant Rat TNF-α (Catalog # 510-RT), and Recombinant Human APRIL/TNFSF13 (Catalog # 5860-AP). PVDF Membrane was probed with 1 µg/mL of Mouse Anti-Human TNF-α Monoclonal Antibody (Catalog # MAB610) followed by HRP-conjugated Anti-Mouse IgG Secondary Antibody (Catalog # HAF007). A specific band was detected for TNF-α at approximately 15 kDa (as indicated). This experiment was conducted under reducing conditions and using Immunoblot Buffer Group 3.

Immunocytochemistry



TNF-α in Human PBMCs. TNF-α was detected in immersion fixed human peripheral blood mononuclear cells (PBMCs) stimulated with LPS and monensin using Mouse Anti-Human TNF-α Monoclonal Antibody (Catalog # MAB610) at 10 µg/mL for 3 hours at room temperature. Cells were stained using the NorthernLights™ 557-conjugated Anti-Mouse IgG Secondary Antibody (yellow; Catalog # NL007) and counterstained with DAPI (blue). View our protocol for Fluorescent ICC Staining of Non-adherent Cells.

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Human TNF-α Antibody

Monoclonal Mouse IgG_1 Clone # 28401

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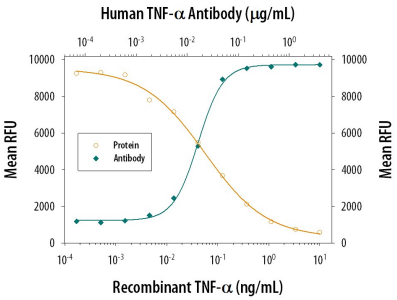
Neutralization



Cytotoxicity Induced by TNF-α and Neutralization by Human TNF-α Antibody. Recombinant Human TNF-α (Catalog # 210-TA) induces cytotoxicity in the the L-929 mouse fibroblast cell line in a dose-dependent manner (orange line). Cytotoxicity elicited by Recombinant Human TNF-α (0.25 ng/mL) is neutralized (green line) by increasing concentrations of Mouse Anti-Human TNF-α Monoclonal Antibody (Catalog # MAB610). The ND_{50} is typically 0.01-0.04 µg/mL in the presence of the metabolic inhibitor actinomycin D.

PREPARATION AND STORAGE

Reconstitution	Reconstitute at 0.5 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-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- is produced by several lymphoid cells as well as by astrocytes, endothelial cells, and smooth muscle cells. Human TNF-alpha consisits of a 35 amino acid (aa) cytoplasmic domain, a 21 aa transmembrane segment, and a 177 aa extracellular domain (ECD). Within the ECD, human TNF-alpha shares 97% aa sequence identity with rhesus and 71%-92% with bovine, canine, cotton rat, equine, feline, mouse, porcine, and rat 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.

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