

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	<i>E. coli</i> -derived recombinant human TNF- α Gly57-Leu233 (predicted) Accession # P01375
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 as a 0.2 μ m filtered solution in PBS.

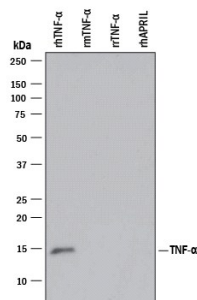
APPLICATIONS

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	1 μ g/mL	See Below
Immunocytochemistry	8-25 μ g/mL	See Below
Human TNF-α Sandwich Immunoassay		Reagent
ELISA Capture	2-8 μ g/mL	Human TNF- α Antibody (Catalog # MAB610)
ELISA Detection Standard	0.1-0.4 μ g/mL	Human TNF- α Biotinylated Antibody (Catalog # BAF210) 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 <i>Lymphokines and Interferons, A Practical Approach</i> . Clemens, M.J. <i>et al.</i> (eds): IRL Press. 221. The Neutralization Dose (ND ₅₀) is typically 0.01-0.04 μ g/mL in the presence of actinomycin D and 0.25 ng/mL Recombinant Human TNF- α .	

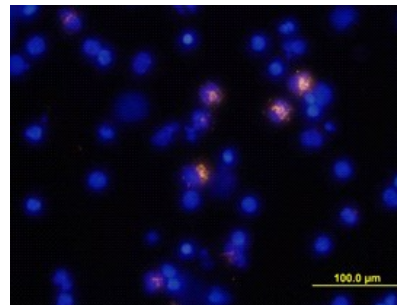
DATA

Western Blot



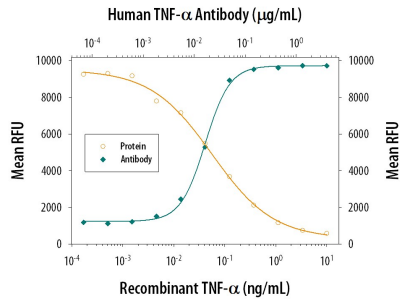
Detection of Recombinant Human TNF- α by Western Blot. Western blot shows 25 ng of Recombinant Human TNF- α (Catalog # [210-TA](#)), Recombinant Mouse TNF- α aa 80-235 (Catalog # [410-MT](#)), Recombinant Rat TNF- α (Catalog # [510-RT](#)), and Recombinant Human APRIL/TNFSF13 (Catalog # [5860-AP](#)). PVDF Membrane was probed with 1 μ g/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 μ g/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](#).

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 ND₅₀ is typically 0.01-0.04 μ g/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. <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

TNF- α , designated TNFSF2, is a trimeric glycoprotein active in both membrane bound and secreted forms. TNF- α is produced by several lymphoid cells as well as by astrocytes, endothelial cells, and smooth muscle cells. TNF- α binds to TNF RI and TNF RII present on virtually all cell types where it triggers the activation of multiple signal transduction pathways and modulates the expression of a wide variety of genes.