

Human Tumor Necrosis Factor- α (hTNF- α)

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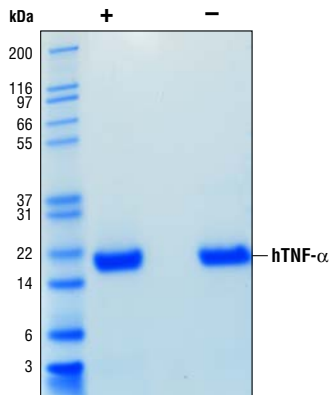
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Source: Recombinant human TNF- α (hTNF- α) Val77-Leu233 (Accession #HUMTNFAB) was produced in *E. coli* at Cell Signaling Technology.

Molecular Characterization: Recombinant hTNF- α does not have a Met on the amino terminus and has a calculated MW of 17,352. DTT-reduced and non-reduced protein migrate as 18 kDa polypeptides. The expected amino-terminal VRSSS of recombinant hTNF- α was verified by amino acid sequencing. TNF- α is a non-disulfide-linked homotrimer in solution as determined by chemical cross-linking.

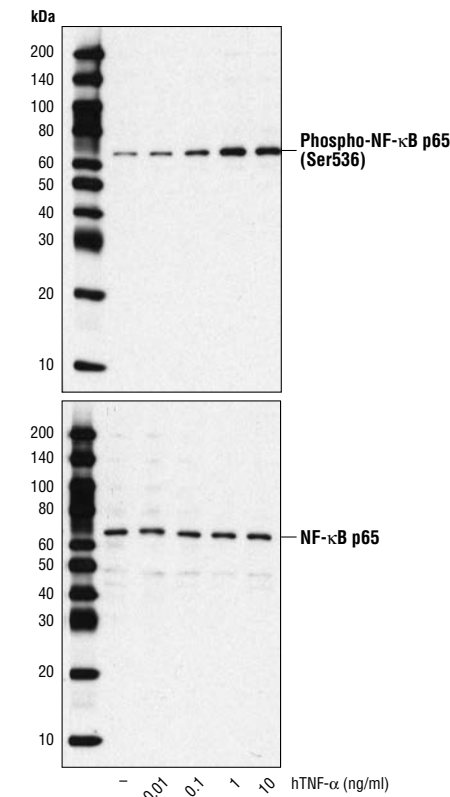
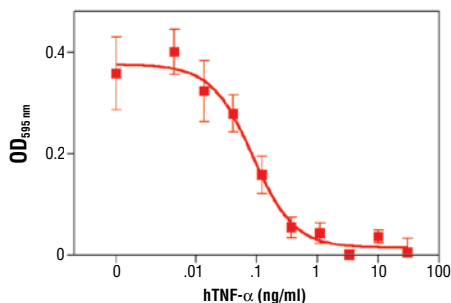
Endotoxin: Less than 0.01 ng endotoxin/1 μ g hTNF- α .

Purity: >98% as determined by SDS-PAGE of 6 μ g reduced (+) and non-reduced (-) recombinant hTNF- α . All lots are greater than 98% pure.



The purity of recombinant hTNF- α was determined by SDS-PAGE of 6 μ g reduced (+) and non-reduced (-) recombinant hTNF- α and staining overnight with Coomassie Blue.

Bioactivity: The bioactivity of hTNF- α was determined in an L-929 cell viability assay. The ED₅₀ of each lot is between 10–500 pg/ml.



Western blot analysis of extracts from HeLa cells treated with hTNF- α for 20 minutes, using Phospho-NF- κ B p65 (Ser536) (93H1) Rabbit mAb #3033 (upper) and total NF- κ B p65 Antibody #3034 (lower).

◀ The viability of L-929 cells treated with increasing amounts of hTNF- α in the presence of 2 ng/ml actinomycin D was determined. Cells were stained with crystal violet at the end of treatment and the OD₅₉₅ was determined.

Formulation: With carrier: Lyophilized from a 0.22 μ m filtered solution of PBS, pH 7.2 containing 20 μ g BSA per 1 μ g hTNF- α .

Carrier free: Lyophilized from a 0.22 μ m filtered solution of PBS, pH 7.2.

Reconstitution:

With carrier: Add sterile PBS, or PBS containing 1% bovine or human serum albumin or 5–10% FBS to a final hTNF- α concentration of greater than 50 μ g/ml. Solubilize for 30 minutes at room temperature with occasional gentle vortexing.

Carrier free: Add sterile PBS, or PBS containing protein to minimize absorption of hTNF- α to surfaces. Solubilize for 30 minutes at room temperature with occasional gentle vortexing. Stock hTNF- α should be greater than 50 μ g/ml.

Storage: Stable in lyophilized state at -20°C for 1 year after receipt. Sterile stock solutions reconstituted with carrier protein are stable at 4°C for 2 months and at -20°C for 6 months. Avoid repeated freeze-thaw cycles.

Maintain sterility. Storage at -20°C should be in a manual defrost freezer.

Applications: Optimal concentration for the desired application should be determined by the user.

Background: TNF- α , the prototypical member of the TNF protein superfamily, is a homotrimeric type-II membrane protein (1,2). Membrane bound TNF- α is cleaved by the metalloprotease TACE/ADAM17 to generate a soluble homotrimer (2). Both membrane and soluble forms of TNF- α are biologically active. TNF- α is produced by a variety of immune cells including T cells, B cells, NK cells and macrophages (1). Cellular response to TNF- α is mediated through interaction with receptors TNF-R1 and TNF-R2 and results in activation of pathways that favor both cell survival and apoptosis depending on the cell type and biological context. Activation of kinase pathways (including JNK, ERK (p44/42), p38 MAPK and NF- κ B) promotes the survival of cells, while TNF- α mediated activation of caspase-8 leads to programmed cell death (1,2). TNF- α plays a key regulatory role in inflammation and host defense against bacterial infection, notably *Mycobacterium tuberculosis* (3). The role of TNF- α in autoimmunity is underscored by blocking TNF- α action to treat rheumatoid arthritis and Crohn's disease (1,2,4).

Background References:

- (1) Aggarwal, B.B. (2003) *Nat Rev Immunol* 3, 745–56.
- (2) Hehlhans, T. and Pfeffer, K. (2005) *Immunology* 115, 1–20.
- (3) Lin, P.L. et al. (2007) *J Invest Dermatol Symp Proc* 12, 22–5.
- (4) Brennan, F.M. and McInnes, I.B. (2008) *J Clin Invest* 118, 3537–45.