Human Tumor Necrosis Factor-α (hTNF-α)

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/μg hTNF-α.

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

Formulation: With carrier. Lyophilized from a 0.22 μm filtered solution of PBS, pH 7.2 containing 20 μg BSA per 1 μg hTNF-α. Carrier free: Lyophilized from a 0.22 μm 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 μg/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 μg/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-κB) 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:

The purity of recombinant hTNF-α was determined by SDS-PAGE of 6 μg 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-κB p65 (Ser536) (93H1) Rabbit mAb #3033 (upper) and total NF-κB 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.