Sterile

#8904

Human Interleukin-6 (hIL-6)

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rev. 03/10/20

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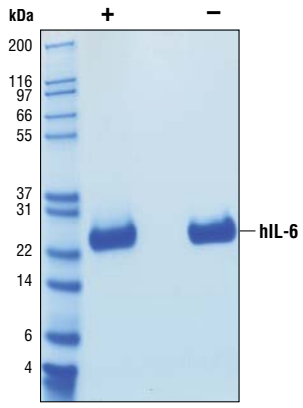
Source: Recombinant human IL-6 (hIL-6) Val30-Met212 (Accession #NM_000600) was produced in *E. coli* at Cell Signaling Technology.

Molecular Characterization: Based on amino acid sequencing, greater than 60% of recombinant hIL-6 has a Met on the amino-terminal Val30 (sequence of MVPPG) and has a calculated MW of 20,943. The remainder starts at pro 32 (sequence of PPGED) or Pro33 (sequence of PGEDS). DTT-reduced protein migrates as a 23 kDa polypeptide and non-reduced protein migrates as a 22 kDa polypeptide due to intramolecular cystines.

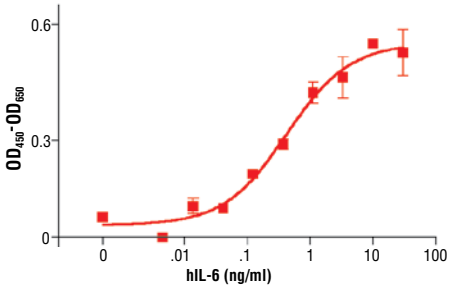
Endotoxin: Less than 0.01 ng endotoxin/1 μg hIL-6.

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

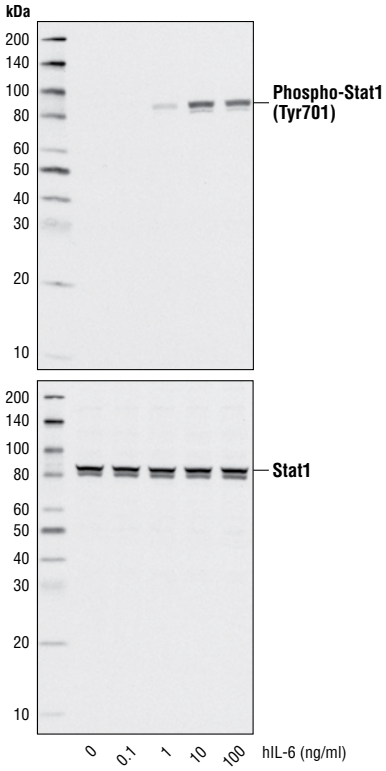


The purity of recombinant hIL-6 was determined by SDS-PAGE of 6 μg reduced (+) and non-reduced (-) recombinant hIL-6 and staining overnight with Coomassie Blue.

Bioactivity: The bioactivity of recombinant hIL-6 was determined in a TF-1 cell proliferation assay. The ED_{50} of each lot is between 0.2-0.7 ng/ml.



◀ *The proliferation of TF-1 cells treated with increasing concentrations of hIL-6 was assessed. After 48 hour treatment with hIL-6, cells were incubated with a tetrazolium salt and the OD_{450} - OD_{650} was determined.*



Western blot analysis of extracts from TF-1 cells, untreated or treated with hIL-6 for 10 minutes, using Phospho-Stat1 (Tyr701) Antibody #9171 (upper) and Stat1 Antibody #9172 (lower).

Formulation: With carrier: Lyophilized from a 0.22 μm filtered solution of PBS, pH 7.2 containing 20 μg BSA per 1 μg hIL-6.

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 hIL-6 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 hIL-6 to surfaces. Solubilize for 30 minutes at room temperature with occasional gentle vortexing. Stock hIL-6 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: IL-6 is a potent inducer of the acute phase response and is produced by T cells, macrophages, fibroblasts, endothelial and other cells (1,2). IL-6 induces proliferation and differentiation and acts on B cells, T cells, thymocytes, and others. IL-6 in concert with TGFβ is important for developing Th17 responses. IL-6 binds to IL-6Rα that through association induces gp130 homodimerization (1). gp130 homodimerization triggers the Jak/STAT cascade and the SHP2/Erk Map kinase cascade (1,3,4). IL-6 also forms a complex with an IL-6Rα splice variant that is non-membrane associated (3).The IL-6/soluble IL-6Rα complex can then activate the gp130 signaling pathway on cells that express gp130 but not IL6Rα (3). IL-6, through increasing expression of proangiogenic VEGF, may contribute to metastatic breast cancer (5).

Background References:

(1) Heinrich, P.C. et al. (1998) *Biochem J* 334 (Pt 2), 297–314.

(2) Heinrich, P.C. et al. (1998) *Z Ernahrungswiss* 37 Suppl 1, 43–9.

(3) Jones, S.A. (2005) *J Immunol* 175, 3463–8.

(4) Jenkins, B.J. et al. (2004) *Mol Cell Biol* 24, 1453–63.

(5) Hong, D.S. et al. (2007) *Cancer* 110, 1911–28.