Sterile

#5216

Mouse Interleukin-6 (mIL-6)

Cell Signaling TECHNOLOGY®

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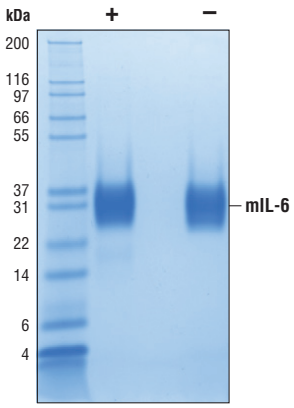
Source: Recombinant mouse IL-6 (mIL-6) Phe25-Thr211 (Accession #NP_112445) was expressed in human 293 cells at Cell Signaling Technology.

Molecular Characterization: Recombinant mIL-6 contains no "tags" and the nonglycosylated protein has a calculated MW of 21,734. DTT-reduced and non-reduced protein migrate as 31 kDa polypeptides. Lower mobility in SDS-PAGE is due to glycosylation. The expected amino-terminal FPTSQ of recombinant mIL-6 was verified by amino acid sequencing.

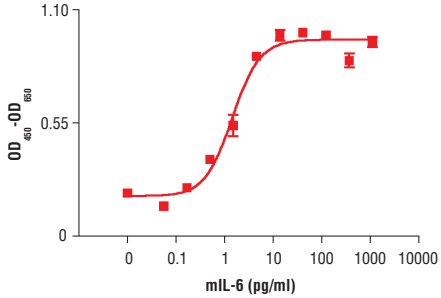
Endotoxin: Less than 0.01 ng endotoxin/1 µg mIL-6.

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

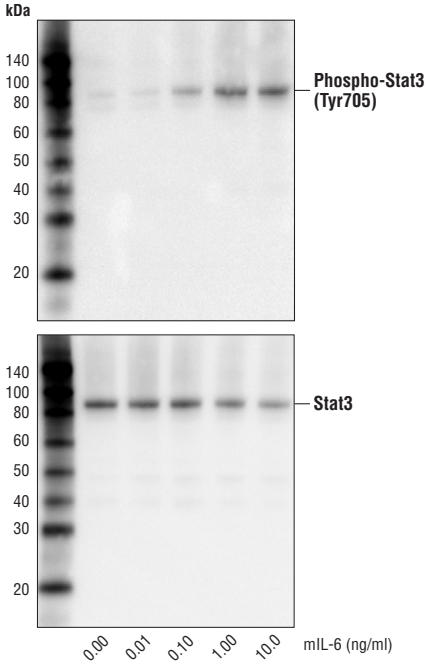


The purity of recombinant mIL-6 was determined by SDS-PAGE of 6 µg reduced (+) and non-reduced (-) recombinant mIL-6 and staining overnight with Coomassie Blue.

Bioactivity: The bioactivity of recombinant mIL-6 was determined in a B9 cell proliferation assay. The ED_{50} of each lot is between 0.5 and 2 pg/ml.



The proliferation of B9 cells treated with increasing concentrations of mIL-6 was assessed. After 48 hour treatment with mIL-6, cells were incubated with a tetrazolium salt and the OD_{450} - OD_{650} was determined.



Western blot analysis of extracts from B9 cells, untreated or treated with mIL-6 for 10 minutes, using Phospho-Stat3 (Tyr705) (D3A7) XP® Rabbit mAb #9145 (upper) and Stat3 (79D7) Rabbit mAb #4904 (lower).

Formulation: With carrier: Lyophilized from a 0.22 µm filtered solution of PBS, pH 7.2 containing 20 µg BSA per 1 µg mIL-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 mIL-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 mIL-6 to surfaces. Solubilize for 30 minutes at room temperature with occasional gentle vortexing. Stock mIL-6 should be greater than 50 µg/ml.

Storage: Stable in lyophilized state at 4°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 and through this association induces gp130 homodimerization (1). gp130 homodimerization triggers the Jak/Stat cascade and the SHP2/MAPK (Erk) 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 IL-6Rα (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.