

Human Interleukin-4 (hIL-4)

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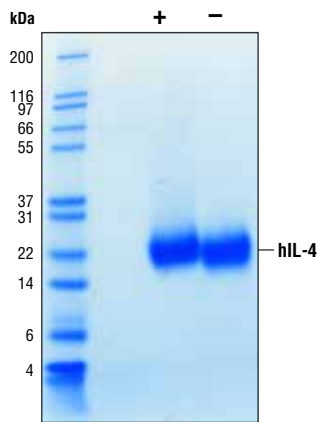
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Source: Recombinant human IL-4 (hIL-4) His25-Ser153 (Accession #AF395008) was expressed in human 293 cells at Cell Signaling Technology.

Molecular Characterization: Recombinant hIL-4 does not have a Met on the amino terminus and the nonglycosylated protein has a calculated MW of 14,963. DTT-reduced and non-reduced protein migrate as larger 20 kDa polypeptides due to glycosylation, with non-reduced having slightly greater mobility due to an intramolecular cystine. The expected amino-terminal HKCDI of recombinant hIL-4 was verified by amino acid sequencing.

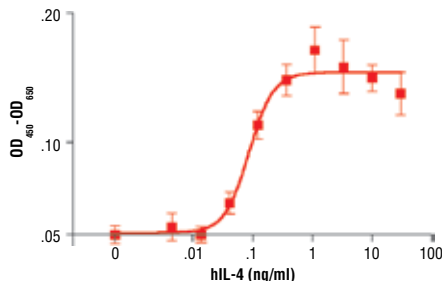
Endotoxin: Less than 0.01 ng endotoxin/1 μ g hIL-4.

Purity: >98% as determined by SDS-PAGE of 6 μ g reduced (+) and non-reduced (-) recombinant hIL-4. All lots are greater than 98% pure.

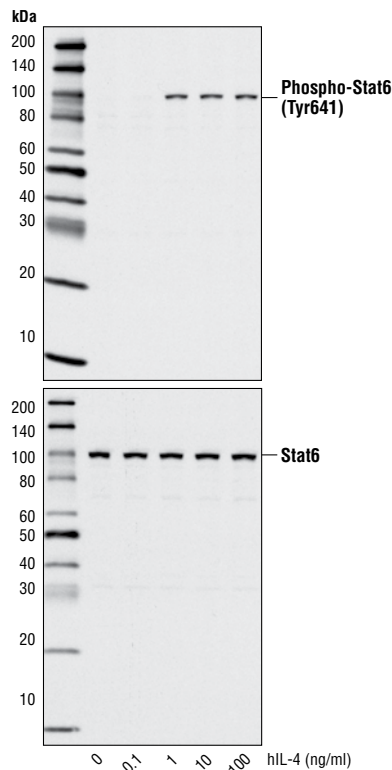


The purity of recombinant hIL-4 was determined by SDS-PAGE of 6 μ g reduced (+) and non-reduced (-) recombinant hIL-4 and staining overnight with Coomassie Blue.

Bioactivity: The bioactivity of recombinant hIL-4 was determined in a TF-1 cell proliferation assay. The ED₅₀ of each lot is between 80-250 pg/ml.



The proliferation of TF-1 cells treated with increasing concentrations of hIL-4 was assessed. After 48 hour treatment with hIL-4, cells were incubated with a tetrazolium salt and the OD₄₅₀ - OD₆₅₀ was determined.



Western blot analysis of extracts from TF-1 cells, untreated or treated with hIL-4 for 20 minutes, using Phospho-Stat6 (Tyr641) (C11A12) Rabbit mAb Antibody #9364 (upper) and Stat6 Antibody #9362 (lower).

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 hIL-4.

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 hIL-4 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 hIL-4 to surfaces. Solubilize for 30 minutes at room temperature with occasional gentle vortexing. Stock hIL-4 should be greater than 50 μ g/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-4 is produced by T cells, NK T cells, $\gamma\delta$ cells, and mast cells (1). Target cells include B cells, T cells, and macrophages (1). IL-4 induces differentiation of naive T cells into the TH2 phenotype. IL-4 also promotes B cell proliferation, antibody isotype switching and expression of other TH2 cytokines including IL-5 and IL-9. IL-4 induced TH2 polarization is important in developing humoral immunity against extracellular pathogens (1) and is involved in the development of allergy and asthma (2). IL-4 binds to two distinct receptors, the type I receptor and type II receptor. Type I receptor is a heterodimer consisting of IL-4R α chain and the common gamma chain, γ c (3,4). Type II receptor, which is shared with IL-13, is a heterodimer of IL-4R α and IL-13R α 1. Signaling initiated via type I receptor results in the activation of Jak1/Stat6, Jak3 and the PI3K/Akt pathways (3). The type II receptor activates the Jak1/Stat6 and the Tyk2/Stat3 pathways (3).

Background References:

- (1) Corthay, A. (2006) *Scand J Immunol* 64, 93-6.
- (2) Nakajima, H. and Takatsu, K. (2007) *Int Arch Allergy Immunol* 142, 265-73.
- (3) Wills-Karp, M. and Finkelman, F.D. (2008) *Sci Signal* 1, pe55.
- (4) Mueller, T.D. et al. (2002) *Biochim Biophys Acta* 1592, 237-50.