Sterile

#8920

Human Interleukin-21 (hIL-21)

Cell Signaling TECHNOLOGY®

Orders ■ 877-616-CELL (2355)
orders@cellsignal.com
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info@cellsignal.com
Web ■ www.cellsignal.com

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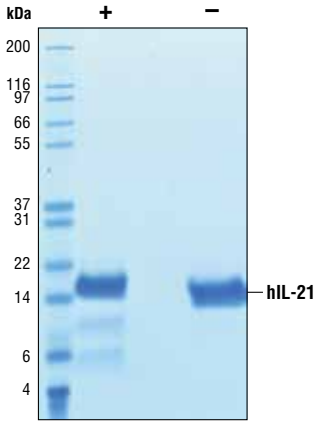
Source: Recombinant human IL-21 (hIL-21) Gln30-Ser155 (Accession # NM_021803) was expressed in human 293 cells at Cell Signaling Technology.

Molecular Characterization: Recombinant hIL-21 contains no "tags" and the nonglycosylated protein has a calculated MW of 15,463. DTT-reduced and non-reduced protein migrate as 16 kDa polypeptides, with non-reduced having slightly greater mobility due to intramolecular cystines. The expected amino-terminal QGQDR of recombinant hIL-21 was verified by amino acid sequencing.

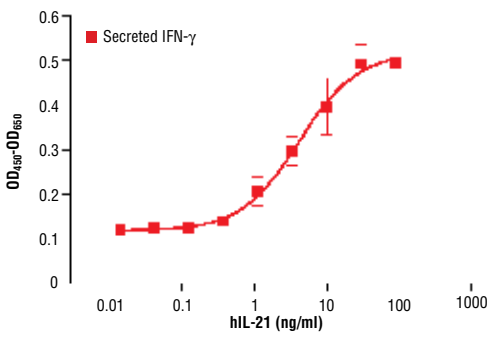
Endotoxin: Less than 0.01 ng endotoxin/1 μg hIL-21.

Purity: >98% as determined by SDS-PAGE of 6 μg reduced (+) and non-reduced (-) recombinant hIL-21. Less than 9% is nicked after Arg114 to generate 6 kDa and 10 kDa polypeptides observed in reduced (+) hIL-21.

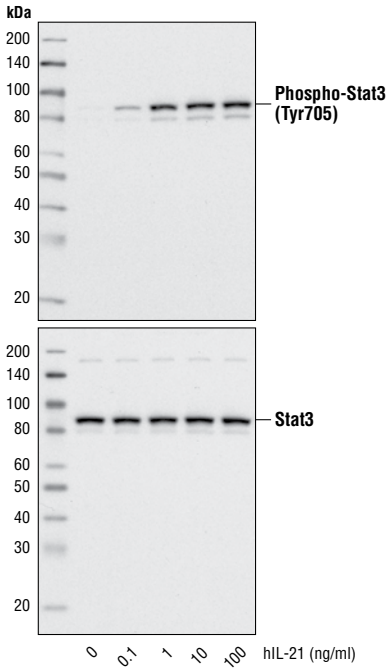


The purity of recombinant hIL-21 was determined by SDS-PAGE of 6 μg reduced (+) and non-reduced (-) recombinant hIL-21 and staining overnight with Coomassie Blue.

Bioactivity: The bioactivity of recombinant hIL-21 was determined by its ability to induce IFN-γ production by NK-92 cells. The ED_{50} of each lot is between 1.0-6.0 ng/ml.



The production of IFN-γ by NK-92 cells cultured with increasing concentrations of hIL-21 was assessed. Media from cells incubated with hIL-21 for 24 hours was collected and assayed for IFN-γ by ELISA and the OD_{450}-OD_{650} was determined.



Western blot analysis of extracts from NK-92 cells, untreated or treated with hIL-21 for 15 minutes, using Phospho-Stat3 (Tyr705) (D3A7) XP™ Rabbit mAb #9145 (upper) or Stat3 Antibody #9132 (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-21.

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-21 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-21 to surfaces. Solubilize for 30 minutes at room temperature with occasional gentle vortexing. Stock hIL-21 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-21 is produced by Th17, T follicular helper (Tfh) and NKT cells (1–3). Targets include T cells, B cells, NK cells and dendritic cells (1–3,5). IL-21 induces proliferation and activation of NK cells, thereby up-regulating IFN-γ production and cytotoxic activity (1,3,4). IL-21 increases proliferation and survival of CD40 primed B cells (1,2) and appears to have a significant role in plasma cell differentiation (2). IL-21 binds to a complex consisting of IL-21Rα and the common γ chain, γc. IL-21 binding activates the Jak1/Jak3, Stat1, Stat3 and Stat5 pathway. IL-21 binding can also activate the MAP kinase and PI3K/Akt pathways.

Background References:

(1) Parrish-Novak, J. et al. (2000) *Nature* 408, 57–63.

(2) Parrish-Novak, J. et al. (2002) *J Leukoc Biol* 72, 856–63.

(3) Konforte, D. et al. (2009) *J Immunol* 182, 1781–7.

(4) Strengell, M. et al. (2002) *J Immunol* 169, 3600–5.

(5) Davis, I.D. et al. (2007) *Clin Cancer Res* 13, 6926–32.