

Human Granulocyte Colony Stimulating Factor (hG-CSF)

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rev. 04/13/17

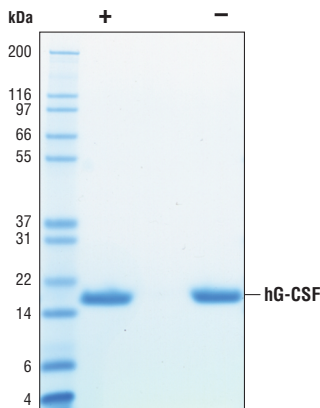
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Source: Recombinant human G-CSF (hG-CSF) Thr31-Pro204 (Accession #NP_757373) was expressed in human 293 cells at Cell Signaling Technology.

Molecular Characterization: Recombinant hG-CSF contains no "tags" and the nonglycosylated protein has a calculated MW of 18,986. DTT-reduced and non-reduced protein migrate as 18 kDa polypeptides. The expected amino-terminal TPLGP of recombinant hG-CSF was verified by amino acid sequencing.

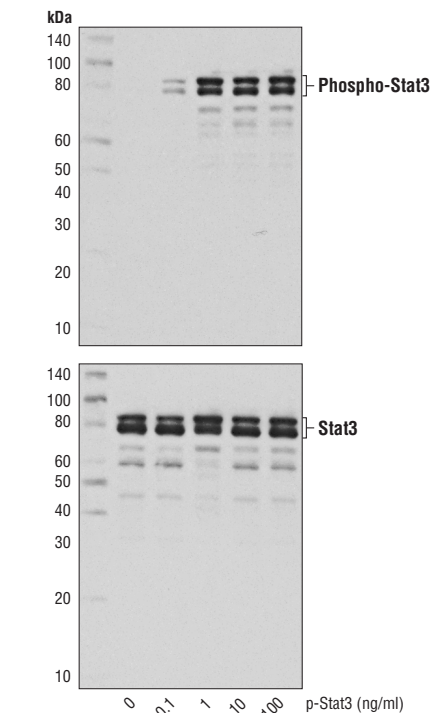
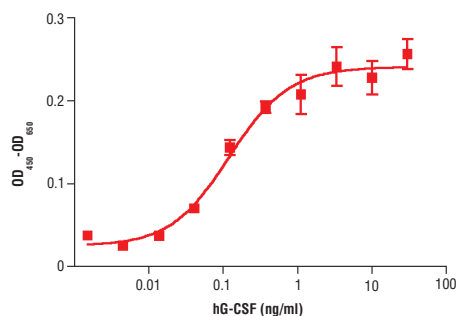
Endotoxin: Less than 0.01 ng endotoxin/1 μ g hG-CSF.

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



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

Bioactivity: The bioactivity of recombinant hG-CSF was determined in a M-NFS-60 cell proliferation assay. The ED₅₀ of each lot is between 20-150 pg/ml.



Western blot analysis of extracts from M-NFS-60 cells untreated or treated with hG-CSF for 15 minutes, using Phospho-Stat3 (Tyr705) (D3A7) Rabbit mAb #9145 (upper) or Stat3 Antibody #9132 (lower).

◀ The proliferation of M-NFS-60 cells treated with increasing concentrations of hG-CSF was assessed. After 72 hour treatment with hG-CSF, cells were incubated with a tetrazolium salt and the OD₄₅₀ - OD₆₅₀ was determined.

Formulation: With carrier: Lyophilized from a 0.22 μ m filtered solution of 40 mM phosphate pH 4.0 containing 250 mM NaCl and 20 μ g BSA per 1 μ g hG-CSF.

Carrier free: Lyophilized from a 0.22 μ m filtered solution of 40 mM phosphate pH 4.0 containing 250 mM NaCl.

Reconstitution:

With carrier: Add sterile 40 mM phosphate pH 4.0 to a final hG-CSF concentration of greater than 50 μ g/ml. Solubilize for 30 minutes at room temperature with occasional gentle vortexing.

Carrier free: Add sterile 40 mM phosphate pH 4.0, or 40 mM phosphate pH 4.0 containing protein to minimize absorption of hG-CSF to surfaces. Solubilize for 30 minutes at room temperature with occasional gentle vortexing. Stock hG-CSF 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: G-CSF is a hematopoietic cytokine essential for neutrophil development, survival, and egress from bone marrow (1-4). Macrophages and monocytes are the predominant producers of G-CSF (3) and endothelial cells, fibroblasts and neuronal cells can produce G-CSF in response to inflammatory stimuli (3). G-CSF inhibits apoptosis in neutrophils and neurons (4,5). G-CSF stimulates proliferation and differentiation of neuronal progenitor cells (5). G-CSF binding to G-CSFR induces receptor dimerization and activation of Jak1/2 tyrosine phosphorylation (3,6). Signaling is through Stat3, ERK, p38, and Akt (5,6). Absence of functional G-CSF or its receptor in humans and mice causes neutropenia (7,8).

Background References:

- (1) Furze, R.C. and Rankin, S.M. (2008) *Immunology* 125, 281-8.
- (2) Demetri, G.D. and Griffin, J.D. (1991) *Blood* 78, 2791-808.
- (3) Srinivasa, S.P. and Doshi, P.D. (2002) *Leukemia* 16, 244-53.
- (4) van Raam, B.J. et al. (2008) *Blood* 112, 2046-54.
- (5) Schneider, A. et al. (2005) *J Clin Invest* 115, 2083-98.
- (6) Nicholson, S.E. et al. (1994) *Proc Natl Acad Sci U S A* 91, 2985-8.
- (7) Lieschke, G.J. et al. (1994) *Blood* 84, 1737-46.
- (8) Dong, F. et al. (1994) *Proc Natl Acad Sci U S A* 91, 4480-4.