Sterile

#8922

Human Granulocyte Macrophage Colony Stimulating Factor (hGM-CSF)

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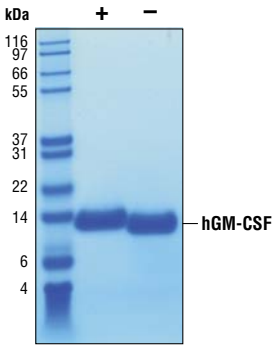
For Research Use Only. Not For Use In Diagnostic Procedures.

Source: Recombinant human GM-CSF (hGM-CSF) Ala18 – Glu144 (Accession # NM_000758) was produced in *E. coli* at Cell Signaling Technology.

Molecular Characterization: Recombinant hGM-CSF does not have a Met on the amino terminus and has a calculated MW of 14477. DTT-reduced protein migrates as a 14 kDa polypeptide and non-reduced protein has slightly greater mobility due to intramolecular cystines. The expected amino-terminal APARS of recombinant hGM-CSF was verified by amino acid sequencing.

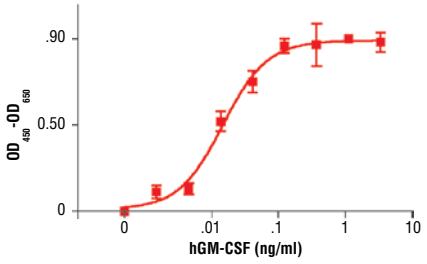
Endotoxin: Less than 0.01 ng endotoxin/1μg hGM-CSF.

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

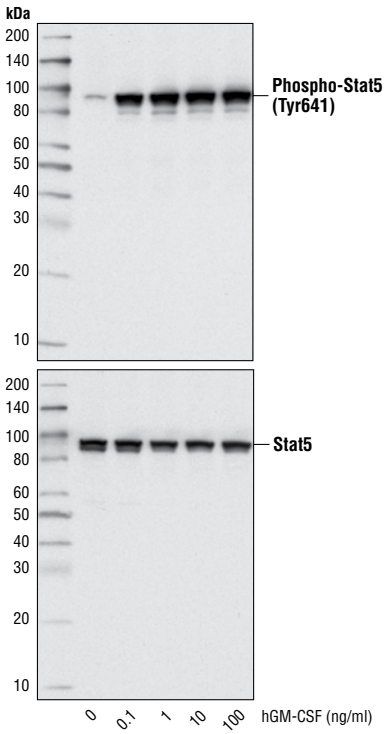


The purity of recombinant hGM-CSF was determined by SDS-PAGE of 6 μg reduced (+) and non-reduced (-) recombinant hGM-CSF and staining overnight with Coomassie Blue.

Bioactivity: The bioactivity of recombinant hGM-CSF was determined in a TF-1 cell proliferation assay. The ED_{50} of each lot is between 5-500 pg/ml.



The proliferation of TF-1 cells treated with increasing concentrations of hGM-CSF was assessed. After 48 hour treatment with hGM-CSF, 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 hGM-CSF for 10 minutes, using Phospho-Stat5 (Tyr694) (C11C5) Rabbit mAb #9359 (upper) and Stat5 (3H7) Rabbit mAb #9358 (lower).

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

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 hGM-CSF 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 hGM-CSF to surfaces. Solubilize for 30 minutes at room temperature with occasional gentle vortexing. Stock hGM-CSF 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: GM-CSF is produced by activated T cells, NK cells and macrophages (1,5). Target cells include granulocyte, monocyte precursors and subsets of differentiated myeloid cells (1,2,3). Many target cells require GM-CSF for survival. GM-CSF induces proliferation, is involved in hematopoietic differentiation of dendritic cells and is a key factor in differentiation pathways leading from stem cells. GM-CSF activates effector functions of myeloid cells, thereby linking adaptive and innate immunity and in turn may boost anti-tumor immunity (4). GM-CSF receptor is composed of GM-CSFRα and the common β chain, βC, which is also utilized by IL-3 and IL-5 (1). Binding of GM-CSF initiates the Jak2, Stat5 and PI3K/Akt pathways (1).

Background References:

(1) Guthridge, M.A. et al. (1998) *Stem Cells* 16, 301–13.

(2) Sonoda, Y. et al. (1988) *Proc Natl Acad Sci USA* 85, 4360–4.

(3) Sonoda, Y. et al. (1988) *Blood* 72, 1381–6.

(4) de la Cruz-Merino, L. et al. (2008) *Oncologist* 13, 1246–54.

(5) Zhang, A.L. et al. (2007) *Blood* 110, 2484–93.