

Human Macrophage Colony Stimulating Factor (hM-CSF)

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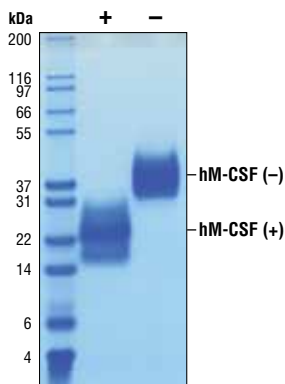
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Source: Recombinant human M-CSF (hM-CSF) Glu33-Ser190 (Accession #P09603-3) was expressed in human 293 cells at Cell Signaling Technology.

Molecular Characterization: Recombinant hM-CSF contains no "tags" and the nonglycosylated protein has a calculated MW of 18,403. DTT-reduced protein migrates as a 16-26 kDa polypeptide and the non-reduced cysteine-linked homodimer migrates as a 34-40 kDa protein. Heterogeneity in SDS PAGE is due to glycosylation. The expected amino-terminal EEVSE of recombinant hM-CSF was verified by amino acid sequencing.

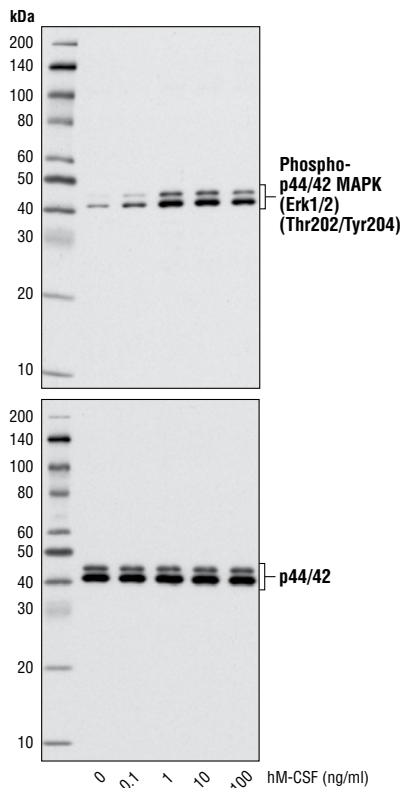
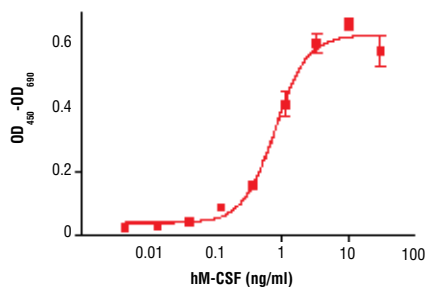
Endotoxin: Less than 0.01 ng endotoxin/1 µg hM-CSF.

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



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

Bioactivity: The bioactivity of recombinant hM-CSF was determined in a M-NFS-60 cell proliferation assay. The ED₅₀ of each lot is between 0.5-15.0 ng/ml.



Western blot analysis of extracts from M-NFS-60 cells, untreated or treated with hM-CSF for 10 minutes, using Phospho-p44/42 MAPK (Erk1/2) (Thr202/Tyr204) (D13.14.4E) XP™ Rabbit mAb #4370 (upper) and p44/42 MAPK (Erk1/2) (137F5) Rabbit mAb #4695 (lower).

◀ The proliferation of M-NFS-60 cells treated with increasing concentrations of hM-CSF was assessed. After 48 hour treatment with hM-CSF, cells were incubated with a tetrazolium salt and the OD₄₉₀ - OD₆₅₀ was determined.

Formulation: With carrier: Lyophilized from a 0.22 µm filtered solution of PBS, pH 7.2 containing 20 µg BSA per 1 µg hM-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 hM-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 hM-CSF to surfaces. Solubilize for 30 minutes at room temperature with occasional gentle vortexing. Stock hM-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: Macrophage-colony stimulating factor (M-CSF) is produced by fibroblasts, endothelial cells, stromal cells, macrophages, osteoblasts and other cell types (1). M-CSF is required for growth and differentiation of monocytes and macrophages (1,2). M-CSF polarizes macrophages into the M2 phenotype where anti-inflammatory IL-10 is produced, rather than the M1 phenotype where inflammatory cytokines are produced. M-CSF also recruits monocytes and enhances angiogenesis by inducing VEGF production (1,2). M-CSF binds to its receptor (CSF1R); downstream signaling involves PI3K/Akt, ERK and STATs 1, 3, and 5 (1,3). An increase in M-CSF expression may contribute to cancer progression and a higher plasma M-CSF level is associated with rheumatoid arthritis (1,4,5).

Background References:

- (1) Hamilton, J.A. (2008) *Nat Rev Immunol* 8, 533-44.
- (2) Curry, J.M. et al. (2008) *PLoS One* 3, e3405.
- (3) Hamilton, J.A. (1997) *J Leukoc Biol* 62, 145-55.
- (4) Rioja, I. et al. (2008) *Arthritis Rheum* 58, 2257-67.
- (5) Skrzypski, M. et al. (2008) *Clin Cancer Res* 14, 4794-9.