Sterile

#5228

Mouse Macrophage Colony Stimulating Factor (mM-CSF)

Cell Signaling TECHNOLOGY®

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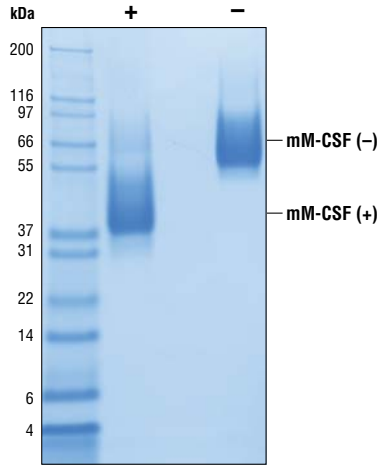
For Research Use Only. Not For Use In Diagnostic Procedures.

Source: Recombinant mouse M-CSF (mM-CSF) Lys33-Glu262 (Accession #NP_031804) was expressed in human 293 cells at Cell Signaling Technology.

Molecular Characterization: Recombinant mM-CSF contains no "tags" and the nonglycosylated protein has a calculated MW of 25,987. DTT-reduced protein migrates as a 37-50 kDa polypeptide and the non-reduced cystine-linked homodimer migrates as a 55-80 kDa protein. Heterogeneity and lower mobility in SDS-PAGE are due to glycosylation. The expected amino-terminal KEVSE of recombinant mM-CSF was verified by amino acid sequencing.

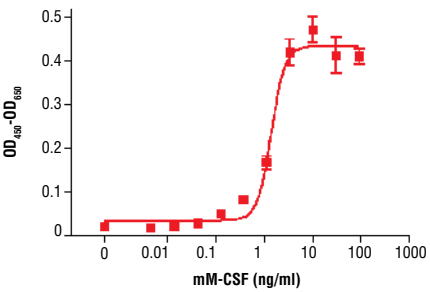
Endotoxin: Less than 0.01 ng endotoxin/1 µg mM-CSF.

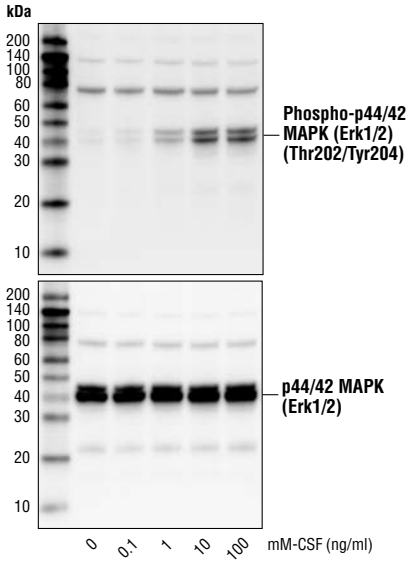
Purity: 98% as determined by SDS-PAGE of 6 µg reduced (+) and non-reduced (-) recombinant mM-CSF. All lots are greater than 98% pure.



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

Bioactivity: The bioactivity of recombinant mM-CSF was determined in an M-NFS-60 cell proliferation assay. The ED50 of each lot is between 0.5-2.5 ng/ml





Western blot analysis of extracts from M-NFS-60 cells, untreated or treated with mM-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 mM-CSF was assessed. After 48 hour treatment with mM-CSF, cells were incubated with a tetrazolium salt and the OD_{450} - OD_{650} 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 mM-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 mM-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 mM-CSF to surfaces. Solubilize for 30 minutes at room temperature with occasional gentle vortexing. Stock mM-CSF should be greater than 50 µg/ml.

Storage: Stable in lyophilized state at -20°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 high plasma M-CSF levels are 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.