

DESCRIPTION

Source	<i>E. coli</i> -derived human M-CSF protein Glu33-Ser190, with an N-terminal Met Accession # NP_757350.2 Produced using non-animal reagents in an animal-free laboratory. Manufactured and tested under cGMP guidelines.
N-terminal Sequence Analysis	Met-Glu-Glu-Val-Ser-Glu-Try-(Cys)-Ser-His
Structure / Form	Disulfide-linked homodimer
Predicted Molecular Mass	18.5 kDa (monomer)

SPECIFICATIONS

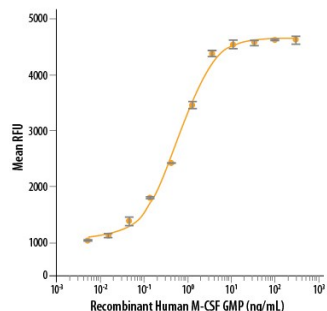
SDS-PAGE	37 kDa, non-reducing conditions
Activity	Measured in a cell proliferation assay using M-NFS-60 mouse myelogenous leukemia lymphoblast cells. Nakoinz, I. <i>et al.</i> (1990) J. Immunol. 145:860. The ED ₅₀ for this effect is 0.5-1.5 ng/mL. The specific activity of Recombinant Human M-CSF is >6.0 x 10 ⁷ IU/mg, which is calibrated against the human M-CSF WHO International Standard (NIBSC code: 89/512).
Endotoxin Level	<0.01 EU per 1 µg of the protein by the LAL method.
Purity	>97%, by SDS-PAGE with silver staining, under reducing conditions.
Host Cell Protein	< 0.5 ng per µg of protein when tested by ELISA.
Mycoplasma	Negative when tested in a ribosomal RNA hybridization assay.
Formulation	Lyophilized from a 0.2 µm filtered solution in PBS. See Certificate of Analysis for details.

PREPARATION AND STORAGE

Reconstitution	Reconstitute at 50-500 µg/mL in PBS.
Shipping	The product is shipped with polar packs. Upon receipt, store it immediately at the temperature recommended below.
Stability & Storage	Use a manual defrost freezer and avoid repeated freeze-thaw cycles. <ul style="list-style-type: none"> • A minimum of 12 months when stored at ≤ -20 °C as supplied. Refer to lot specific COA for the Use by Date. • 1 month, 2 to 8 °C under sterile conditions after reconstitution. • 3 months, ≤ -20 °C under sterile conditions after reconstitution.

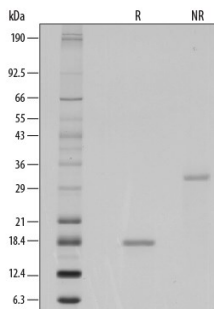
DATA

Bioactivity



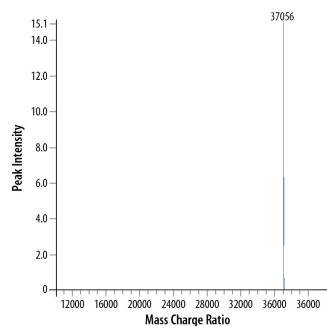
Recombinant Human M-CSF GMP Protein Bioactivity GMP-grade Recombinant Human M-CSF (Catalog # 216-GMP) stimulates cell proliferation in the M-NFS-60 mouse myelogenous leukemia lymphoblast cell line in a dose-dependent manner. The ED₅₀ for this effect is 0.5-1.5 ng/mL.

SDS-PAGE



Recombinant Human M-CSF GMP Protein SDS-PAGE 1 µg/lane of GMP-grade Recombinant Human M-CSF (Catalog # 216-GMP) was resolved with SDS-PAGE under reducing (R) and non-reducing (NR) conditions and visualized by silver staining, showing bands at 18 kDa and 32 kDa, respectively.

Mass Spectrometry



Recombinant Human M-CSF GMP Protein Mass Spectrometry ESI analysis of GMP-grade Recombinant Human M-CSF (Catalog # 216-GMP). The peak at 37056 Da corresponds to the measured molecular weight of the intact dimer. The calculated mass for the monomer is 18535 Da.

BACKGROUND

M-CSF, also known as CSF-1, is a four- α -helical-bundle cytokine that is the primary regulator of macrophage survival, proliferation and differentiation (1-3). M-CSF is also essential for the survival and proliferation of osteoclast progenitors (1, 4). M-CSF also primes and enhances macrophage killing of tumor cells and microorganisms, regulates the release of cytokines and other inflammatory modulators from macrophages, and stimulates pinocytosis (2, 3). M-CSF increases during pregnancy to support implantation and growth of the decidua and placenta (5). Sources of M-CSF include fibroblasts, activated macrophages, endometrial secretory epithelium, bone marrow stromal cells and activated endothelial cells (1-5). The M-CSF receptor (*c-fms*) transduces its pleiotropic effects and mediates its endocytosis. M-CSF mRNAs of various sizes occur (3-9). Full length human M-CSF transcripts encode a 522 amino acid (aa) type I transmembrane (TM) protein with a 464 aa extracellular region, a 21 aa TM domain, and a 37 aa cytoplasmic tail that forms a 140 kDa covalent dimer. Differential processing produces two proteolytically cleaved, secreted dimers. One is an N- and O- glycosylated 86 kDa dimer, while the other is modified by both glycosylation and chondroitin-sulfate proteoglycan (PG) to generate a 200 kDa subunit. Although PG-modified M-CSF can circulate, it may be immobilized by attachment to type V collagen (8). Shorter transcripts encode M-CSF that lacks cleavage and PG sites and produces an N-glycosylated 68 kDa TM dimer and a slowly produced 44 kDa secreted dimer (7). Although forms may vary in activity and half-life, all contain the N-terminal 150 aa portion that is necessary and sufficient for interaction with the M-CSF receptor (10, 11). The first 223 aa of mature human M-CSF shares 88%, 86%, 81% and 74% aa identity with corresponding regions of dog, cow, mouse and rat M-CSF, respectively (12, 13). Human M-CSF is active in the mouse, but mouse M-CSF is reported to be species-specific.

References:

1. Pixley, F.J. and E.R. Stanley (2004) Trends Cell Biol. **14**:628.
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3. Fixe, P. and V. Praloran (1997) Eur. Cytokine Netw. **8**:125.
4. Ryan, G.R. et al. (2001) Blood **98**:74.
5. Makrigiannakis, A. et al. (2006) Trends Endocrinol. Metab. **17**:178.
6. Nandi, S. et al. (2006) Blood **107**:786.
7. Rettenmier, C.W. and M.F. Roussel (1988) Mol. Cell Biol. **8**:5026.
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9. Manos, M.M. (1988) Mol. Cell. Biol. **8**:5035.
10. Koths, K. (1997) Mol. Reprod. Dev. **46**:31.
11. Jang, M-H. et al. (2006) J. Immunol. **177**:4055.
12. Kawasaki, E.S. et al. (1985) Science **230**: 291.
13. Wong, G.G. et al. (1987) Science **235**:1504.

MANUFACTURING SPECIFICATIONS

GMP Proteins

R&D Systems, a Bio-Techne Brand's GMP proteins are produced according to relevant sections of the following documents: USP Chapter 1043, Ancillary Materials for Cell, Gene and Tissue-Engineered Products and Eu. Ph. 5.2.12, Raw Materials of Biological Origin for the Production of Cell-based and Gene Therapy Medicinal Products.

R&D Systems' quality focus includes:

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- Raw material testing and vendor qualification/monitoring
- Fully validated equipment, processes and test methods
- Equipment calibration schedules using a computerized calibration program
- Facility maintenance, safety programs and pest control
- Material review process for variances
- Monitoring of stability over product shelf-life

R&D Systems strives to provide our customers with the analytical characteristics of each product so that customers may determine whether our products are appropriate for their research. The Certificate of Analysis provided contains the following lot specific information:

- N-terminal amino acid analysis, SDS-PAGE analysis, and endotoxin level (as determined by LAL assay) performed on each bulk QC lot, not on individual bottlings of each QC lot
- Post-bottling lot-specific bioassay results (compliance with an established range) and results of microbial testing according to USP
- Host Cell Protein testing performed by ELISA
- Mycoplasma testing by ribosomal RNA hybridization assay

Additional testing and documentation requested by the customer can be arranged at an additional cost.

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- Purified proteins are stored in animal-free containers in a dedicated cold storage room.

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