

DESCRIPTION

Source	<i>E. coli</i> -derived human M-CSF protein Glu33-Ser190, with an N-terminal Met Accession # NP_757350 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.

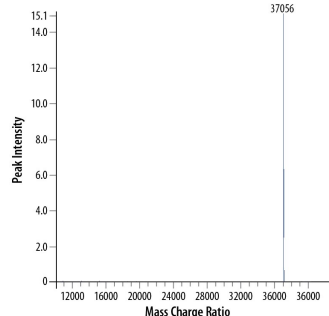
PREPARATION AND STORAGE

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

<p>Bioactivity</p> <p>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.</p>	<p>SDS-PAGE</p> <p>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.</p>
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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 pleotropic 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:

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6. Nandi, S. *et al.* (2006) Blood **107**:786.
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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: WHO TRS, No. 822, 1992 Annex 1, Good Manufacturing Practices for Biological Products; USP Chapter 1043, Ancillary Materials for Cell, Gene and Tissue-Engineered Products and USP Chapter 92, Growth Factors and Cytokines Used in Cell Therapy Manufacturing.

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- 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

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