Quantikine®

Human M-CSF Immunoassay

Catalog Number DMC00
SMC00
PDMC00

For the quantitative determination of human macrophage colony stimulating factor (M-CSF) concentrations in cell culture supernates, serum, and plasma.

This package insert must be read in its entirety before using this product.

FOR RESEARCH USE ONLY.
NOT FOR USE IN DIAGNOSTIC PROCEDURES.

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MANUFACTURED AND DISTRIBUTED BY:

R&D Systems, Inc.
614 McKinley Place NE
Minneapolis, MN 55413
United States of America

TELEPHONE: (800) 343-7475
(612) 379-2956
FAX: (612) 656-4400
E-MAIL: info@RnDSystems.com

DISTRIBUTED BY:

R&D Systems Europe, Ltd.
19 Barton Lane
Abingdon Science Park
Abingdon, OX14 3NB
United Kingdom

TELEPHONE: +44 (0)1235 529449
FAX: +44 (0)1235 533420
E-MAIL: info@RnDSystems.co.uk

R&D Systems China Co. Ltd.
24A1 Hua Min Empire Plaza
726 West Yan An Road
Shanghai PRC 200050

TELEPHONE: +86 (21) 52380373
FAX: +86 (21) 52371001
E-MAIL: info@RnDSystemsChina.com.cn

INTRODUCTION

M-CSF, also known as CSF-1, was originally discovered in serum, urine and other biological fluids as a factor that could stimulate the formation of macrophage colonies from bone marrow hematopoietic progenitor cells (1, 2). M-CSF can be produced by a number of cells, including fibroblasts, secretory epithelial cells of the endometrium, bone marrow stromal cells, brain astrocytes, osteoblasts, renal mesangial cells, keratinocytes and LPS- or cytokine-activated macrophages, B cells, T cells and endothelial cells. A number of human tumors, including myeloblastic leukemias, lymphoblastic leukemias and adenocarcinomas of the lung, breast, ovary and endometrium, have also been shown to produce M-CSF (3, 4). The primary function of M-CSF has been shown to be the regulation of the growth, differentiation and function of mononuclear phagocytes. Studies using osteopetrotic (*op/op*) mutant mice that do not synthesize functional M-CSF, show that the development and function of tissue macrophages that are involved in organogenesis and tissue remodelling are dependent on M-CSF. However, macrophages that are involved in inflammatory and immune responses develop independently of M-CSF (5, 6). M-CSF regulates the differentiation of osteoclast progenitors (5). During pregnancy, a large increase in uterine M-CSF levels has been observed, suggesting a possible role in the formation and differentiation of the placenta (7, 8). M-CSF has been shown to enhance the macrophage uptake and degradation of acetylated LDL and to enhance cholesterol esterification *in vitro*, and administration of M-CSF *in vivo* has also been shown to rapidly lower plasma cholesterol levels in various animal models (3, 5).

Natural M-CSFs purified from both human and mouse sources are disulfide-linked homodimeric glycoproteins and glycosaminoglycan-containing molecules (5). The single copy M-CSF gene has been mapped to human chromosome 1p13-p21 (9) and to mouse chromosome 3 at the *op* locus (10). Several sizes of mRNAs, arising from alternative splicing in both the coding and noncoding regions, have been reported (4, 5). The longest human M-CSF precursor isoform is a 554 amino acid residue Type I transmembrane pre-pro-protein containing a 32 amino acid residue signal peptide followed by an additional 522 amino acid residues (M-CSF^{522}). This protein contains multiple N-linked and O-linked glycosylation sites as well as a glycosaminoglycan addition site. The secreted glycoprotein form of M-CSF is generated from the homodimeric M-CSF^{522} precursor by proteolytic cleavage at the N-terminal side of the glycosaminoglycan addition site. The secreted proteoglycan form of M-CSF is derived from the same precursor by proteolytic cleavage at the C-terminal side of the glycosaminoglycan addition site. These proteolytic cleavage reactions of M-CSF^{522} have been shown to occur intracellularly in the secretory vesicles. In addition to mRNAs encoding M-CSF^{522} precursors, mRNAs encoding shorter M-CSF precursor isoforms (M-CSF^{224} and M-CSF^{406}) have also been identified. The M-CSF^{224} precursor differs from the M-CSF^{522} precursor in that it lacks the proteolytic cleavage site, the O-linked glycosylation site, the glycosaminoglycan addition site and half of the potential N-linked glycosylation sites present in M-CSF^{522}. M-CSF^{224} is expressed as a transmembrane dimeric protein on the cell surface. The generation of soluble M-CSF by proteolysis of M-CSF^{224} has also been demonstrated. The proteolytic cleavage of the membrane-bound M-CSF^{224} is inefficient, but can be stimulated by the activation of protein kinase C (11). A juxtacrine interaction between the membrane-bound M-CSF and the M-CSF receptor is possible. The membrane-bound M-CSF expressed in a fibroblast monolayer has been shown to mediate the adhesion and proliferation of macrophages expressing the M-CSF receptor (12). The N-terminal 150 amino acid residues of the mature M-CSF, a region necessary and sufficient for interaction with the M-CSF R, is highly conserved (80% homology) between the human and mouse proteins. Whereas human M-CSF is active in the mouse system, mouse M-CSF is reported to be species-specific in its action. The three-dimensional structure of soluble M-CSF (containing amino acid residues 4 - 158) has been elucidated, confirming the previously predicted four α-helix bundle structure (13).

M-CSF exerts its pleiotropic effects by binding to a single type of high affinity cell surface receptor that is encoded by the c-*fms* proto-oncogene (5, 14). The gene for human M-CSF receptor has been mapped to human chromosome 5q33.3, linked head to tail with the gene for PDGF Rβ. In the same region of the chromosome, the genes for GM-CSF, IL-3, IL-4, IL-5 and FGF acidic have also been localized (5). Among hematopoietic cells, the highest receptor expression is found on mature circulating monocytes and tissue macrophages. High levels of M-CSF R can also be detected on placental trophoblasts. The human M-CSF receptor is a transmembrane glycoprotein consisting of a 512 amino acid extracellular domain, a single 25 amino acid transmembrane domain and a 435 amino acid intracellular domain. The extracellular region is organized into five immunoglobulin-like domains. The intracellular region contains a tyrosine kinase domain containing kinase insert sequences similar to those found in the SCF gene product and the α and β receptors for PDGF.

Elevated levels of circulating M-CSF have been reported during pregnancy (5) and in patients with systemic lupus erythematosus (15), myeloproliferative diseases, leukemias and lymphoid malignancies (16, 17), ovarian and endometrial cancers (18, 19), immune and pregnancy- associated thrombocytopenia (20), and following infection (21, 22). During bacterial meningitis infection, elevated levels of M-CSF were reported in cerebrospinal fluids (23, 24). M-CSF concentration was also reported to rise following bone marrow transplant and chemotherapy and may be predictive of neutrophil recovery (25). Circulating levels of M-CSF were also reported to be a prognostic indicator in patients with epithelial ovarian cancer (26) and to correlate with clinical response to interferon-α in patients with early-stage B-CLL (27).

The Quantikine Human M-CSF Immunoassay is a 3.5 hour solid phase ELISA designed to measure human M-CSF levels in cell culture supernates, serum, and plasma. It contains *E. coli*-derived recombinant human M-CSF (rhM-CSF^{158}), comprising the amino-terminal 158 amino acid residues of the extracellular domain of native human M-CSF, as well as antibodies raised against this recombinant factor. It has been shown to accurately quantitate rhM-CSF^{158} when calibrated against this recombinant protein. Additionally, linear dose curves were observed when a recombinant human M-CSF (rhM-CSF^{221}), containing 221 amino acid residues (Macrolin® M-CSF obtained from Chiron Corporation), or when natural secreted human M-CSF (from media conditioned by MIA PaCa- or TPA-stimulated HL-60 cells) was used. The dose curves obtained were parallel to the standard curves obtained using the *E. coli*-expressed rhM-CSF^{158}. These results indicate that the Quantikine Human M-CSF Immunoassay kit can be used to determine relative mass values for the long form of recombinant M-CSF and for natural human M-CSF. Assuming that the biological activities of rhM-CSF^{158} and Macrolin M-CSF are equal on a molar basis, it has been found that when equal concentrations of these factors (based on biological potency) were compared, the immunoreactivity of rhM-CSF^{158} was 2.7 fold higher than Macrolin M-CSF.

The composition of naturally occurring M-CSF from differing sources is not known. In order to investigate this question, natural M-CSF preparations from TPA-stimulated HL-60 or MIA PaCa cells, their concentrations normalized on the basis of biological activity, were measured using this immunoassay. These samples exhibited immunoreactivity similar to that of Macrolin M-CSF and lower than that of rhM-CSF^{158}, suggesting that the natural material, at least from these sources, consisted primarily of the longer form of M-CSF. Therefore, to more accurately quantitate M-CSF from natural sources, the Quantikine Human M-CSF Immunoassay is calibrated against the NIBSC/WHO First International Standard for M-CSF (89/512), which is the same as Macrolin M-CSF.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for M-CSF has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any M-CSF present is bound by the immobilized antibody. After washing away any unbound substances, an enzyme-linked polyclonal antibody specific for M-CSF is added to the wells. Following a wash to remove any unbound antibody-enzyme reagent, a substrate solution is added to the wells and color develops in proportion to the amount of M-CSF bound in the initial step. The color development is stopped and the intensity of the color is measured.

LIMITATIONS OF THE PROCEDURE

- FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.
- The kit should not be used beyond the expiration date on the kit label.
- Do not mix or substitute reagents with those from other lots or sources.
- If samples generate values higher than the highest standard, further dilute the samples with the Calibrator Diluent and repeat the assay.
- Any variation in standard diluent, operator, pipetting technique, washing technique, incubation time or temperature, and kit age can cause variation in binding.
- This assay is designed to eliminate interference by soluble receptors, binding proteins, and other factors present in biological samples. Until all factors have been tested in the Quantikine Immunoassay, the possibility of interference cannot be excluded.

MATERIALS PROVIDED

Description	Part #	Cat. # DMC00	Cat. # SMC00
M-CSF Microplate - 96 well polystyrene microplate (12 strips of 8 wells) coated with a mouse monoclonal antibody against M-CSF.	890153	1 plate	6 plates
M-CSF Conjugate - 21 mL/vial of polyclonal antibody against M-CSF conjugated to horseradish peroxidase with preservatives.	890154	1 vial	6 vials
M-CSF Standard - 10 ng/vial of recombinant human M-CSF in a buffered protein base with preservatives; lyophilized.	890155	1 vial	6 vials
Assay Diluent RD1N - 11 mL/vial of a buffered protein base with preservatives. May contain a precipitate. *For cell culture supernate samples.*	895081	1 vial	6 vials
Assay Diluent RD1O - 11 mL/vial of a buffered protein base with preservatives. *For serum/plasma samples.*	895082	1 vial	6 vials
Calibrator Diluent RD6F - 21 mL/vial of animal serum with preservatives.	895018	2 vials	12 vials
Wash Buffer Concentrate - 21 mL/vial of a 25-fold concentrated solution of buffered surfactant with preservatives.	895003	1 vial	6 vials
Color Reagent A - 12.5 mL/vial of stabilized hydrogen peroxide.	895000	1 vial	6 vials
Color Reagent B - 12.5 mL/vial of stabilized chromogen (tetramethylbenzidine).	895001	1 vial	6 vials
Stop Solution - 6 mL/vial of 2 N sulfuric acid.	895032	1 vial	6 vials
Plate Covers - Adhesive strips.	——	4 strips	24 strips

DMC00 contains sufficient materials to run an ELISA on one 96 well plate.
SMC00 (SixPak) contains sufficient materials to run ELISAs on six 96 well plates.

This kit is also available in a PharmPak (R&D Systems, Catalog # PDMC00). PharmPaks contain sufficient materials to run ELISAs on 50 microplates. Specific vial counts of each component may vary. Please refer to the literature accompanying your order for specific vial counts.

STORAGE

<table>
<tr><td>Unopened Kit</td><td colspan="2">Store at 2 - 8° C. Do not use past kit expiration date.</td></tr>
<tr><td rowspan="10">Opened/ Reconstituted Reagents</td><td>Diluted Wash Buffer</td><td rowspan="9">May be stored for up to 1 month at 2 - 8° C.*</td></tr>
<tr><td>Stop Solution</td></tr>
<tr><td>Calibrator Diluent RD6F</td></tr>
<tr><td>Assay Diluent RD1N</td></tr>
<tr><td>Assay Diluent RD1O</td></tr>
<tr><td>Conjugate</td></tr>
<tr><td>Unmixed Color Reagent A</td></tr>
<tr><td>Unmixed Color Reagent B</td></tr>
<tr><td>Standard</td></tr>
<tr><td>Microplate Wells</td><td>Return unused wells to the foil pouch containing the desiccant pack, reseal along entire edge of zip-seal. May be stored for up to 1 month at 2 - 8° C.*</td></tr>
</table>

*Provided this is within the expiration date of the kit.

OTHER SUPPLIES REQUIRED

- Microplate reader capable of measuring absorbance at 450 nm, with the correction wavelength set at 540 nm or 570 nm.
- Pipettes and pipette tips.
- Deionized or distilled water.
- Squirt bottle, manifold dispenser, or automated microplate washer.
- 500 mL graduated cylinder.
- Test tubes for dilution.
- Human M-CSF Controls (optional; available from R&D Systems).

PRECAUTIONS

M-CSF is detectable in saliva. Take precautionary measures to prevent contamination of the kit reagents while running the assay.

Some components of this kit contain sodium azide which may react with lead and copper plumbing to form explosive metallic azides. Flush with large volumes of water during disposal.

Assay Diluent RD1O contains an irritant. Wear eye, hand, face, and clothing protection when using this material.

The Stop Solution provided with this kit is an acid solution. Wear eye, hand, face, and clothing protection when using this material.

SAMPLE COLLECTION AND STORAGE

Cell Culture Supernates - Remove particulates by centrifugation and assay immediately or aliquot and store samples at ≤ -20° C. Avoid repeated freeze-thaw cycles.

Caution: Human serum used in the preparation of cell culture media may contain M-CSF. Because of the low species cross-reactivity of this kit, human M-CSF levels in culture media containing up to 10% bovine or fetal bovine serum can be assayed without interference.

Serum - Use a serum separator tube (SST) and allow samples to clot for 30 minutes before centrifugation for 15 minutes at 1000 x g. Remove serum and assay immediately.

Plasma - Collect plasma using EDTA, heparin, or citrate as an anticoagulant. Centrifuge for 15 minutes at 1000 x g within 30 minutes of collection. Assay immediately.

Note: *It is recommended that fresh serum/plasma samples be used. If it is not possible to use fresh samples, aliquot and store at ≤ -20° C. Avoid repeated freeze/thaw cycles.*

SAMPLE PREPARATION

All cell culture supernate samples require at least a 2-fold dilution into Calibrator Diluent RD6F prior to the assay. A suggested 2-fold dilution is 150 µL Calibrator Diluent RD6F + 150 µL sample.

All serum/plasma samples require at least a 5-fold dilution into Calibrator Diluent RD6F prior to the assay. A suggested 5-fold dilution is 200 µL Calibrator Diluent RD6F + 50 µL sample.

REAGENT PREPARATION

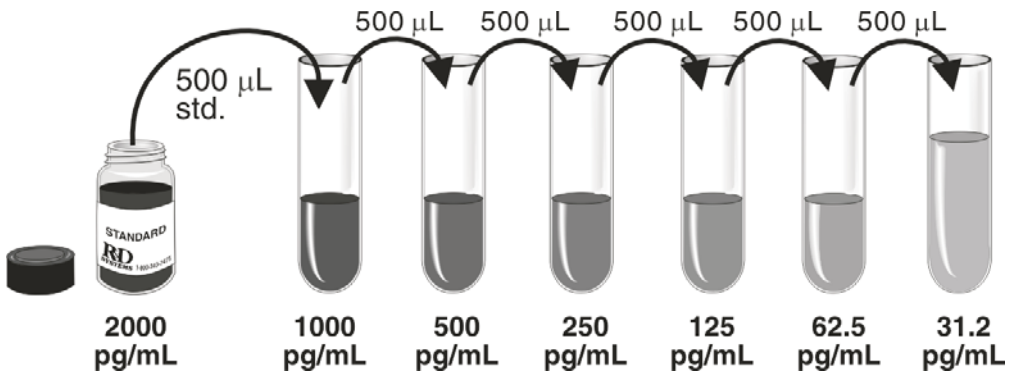
Bring all reagents to room temperature before use.

Wash Buffer - If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. Dilute 20 mL of Wash Buffer Concentrate into deionized or distilled water to prepare 500 mL of Wash Buffer.

Substrate Solution - Color Reagents A and B should be mixed together in equal volumes within 15 minutes of use. Protect from light. 200 µL of the resultant mixture is required per well.

M-CSF Standard - Reconstitute the M-CSF Standard with 5.0 mL of Calibrator Diluent RD6F. This reconstitution produces a stock solution of 2000 pg/mL. Allow the standard to sit for a minimum of 15 minutes with gentle agitation prior to making dilutions.

Pipette 500 µL of Calibrator Diluent RD6F into each tube. Use the stock solution to produce a dilution series (below). Mix each tube thoroughly before the next transfer. The undiluted standard serves as the high standard (2000 pg/mL). Calibrator Diluent RD6F serves as the zero standard (0 pg/mL).



ASSAY PROCEDURE

Bring all reagents and samples to room temperature before use. It is recommended that all samples, standards, and controls be assayed in duplicate.

1. Prepare all reagents, working standards, and samples as directed in the previous sections.

2. Remove excess microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, and reseal.

3. **For Cell Culture Supernate Samples**: Add 100 µL of Assay Diluent RD1N to each well. **For Serum/Plasma Samples**: Add 100 µL of Assay Diluent RD1O to each well.

4. Add 100 µL of Standard, control, or sample* per well. Cover with the adhesive strip provided. Incubate for 1.5 hours at room temperature. A plate layout is provided as a record of samples and standards assayed.

5. Aspirate each well and wash, repeating the process three times for a total of four washes. Wash by filling each well with Wash Buffer (400 µL) using a squirt bottle, manifold dispenser, or autowasher. Complete removal of liquid at each step is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against clean paper towels.

6. Add 200 µL of M-CSF Conjugate to each well. Cover with a new adhesive strip. Incubate for 1.5 hours at room temperature.

7. Repeat the aspiration/wash as in step 5.

8. Add 200 µL of Substrate Solution to each well. Incubate for 30 minutes at room temperature. **Protect from light.**

9. Add 50 µL of Stop Solution to each well. The color in the wells should change from blue to yellow. If the color in the wells is green or the color change does not appear uniform, gently tap the plate to ensure thorough mixing.

10. Determine the optical density of each well within 30 minutes, using a microplate reader set to 450 nm. If wavelength correction is available, set to 540 nm or 570 nm. If wavelength correction is not available, subtract readings at 540 nm or 570 nm from the readings at 450 nm. This subtraction will correct for optical imperfections in the plate. Readings made directly at 450 nm without correction may be higher and less accurate.

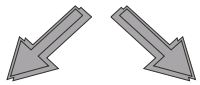
*Serum and plasma samples require at least a 5-fold dilution into Calibrator Diluent RD6F. Cell culture supernate samples require at least a 2-fold dilution into Calibrator Diluent RD6F.

ASSAY PROCEDURE SUMMARY

1. Prepare all reagents, standards and samples as instructed.

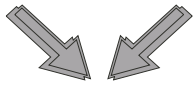
2. Add 100 µL of Assay Diluent to each well.

Cell Culture Supernate Samples



Serum/Plasma Samples

Use Assay Diluent RD1N.



Use Assay Diluent RD1O.

3. Add 100 µL Standard, control, or sample* to each well. Incubate 1.5 hours at RT.

4. Aspirate and wash 4 times.

5. Add 200 µL Conjugate to each well. Incubate 1.5 hours at RT.

6. Aspirate and wash 4 times.

7. Add 200 µL Substrate Solution to each well. Incubate 30 minutes at RT. **Protect from light.**

8. Add 50 µL Stop Solution to each well. Read at 450 nm within 30 minutes. λ correction 540 or 570 nm

*Serum, plasma, and cell culture supernate samples require dilution.

CALCULATION OF RESULTS

Average the duplicate readings for each standard, control, and sample and subtract the average zero standard optical density.

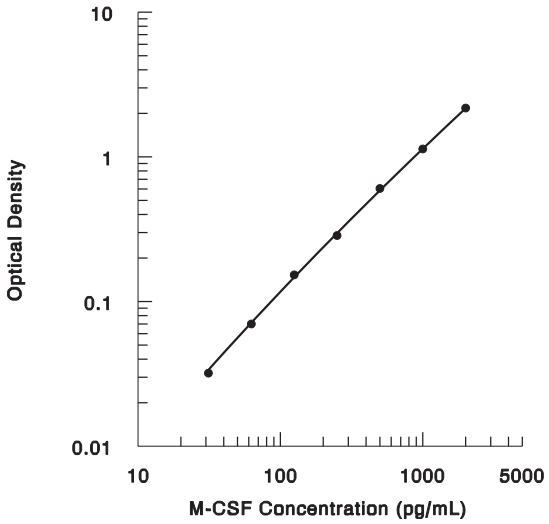
Create a standard curve by reducing the data using computer software capable of generating a four parameter logistic (4-PL) curve-fit. As an alternative, construct a standard curve by plotting the mean absorbance for each standard on the y-axis against the concentration on the x-axis and draw a best fit curve through the points on the graph. The data may be linearized by plotting the log of the M-CSF concentrations versus the log of the O.D. and the best fit line can be determined by regression analysis. This procedure will produce an adequate but less precise fit of the data.

Because culture media, serum, and plasma have been diluted prior to the assay, their measured concentrations must be multiplied by 2, 5, or 5, respectively.

TYPICAL DATA

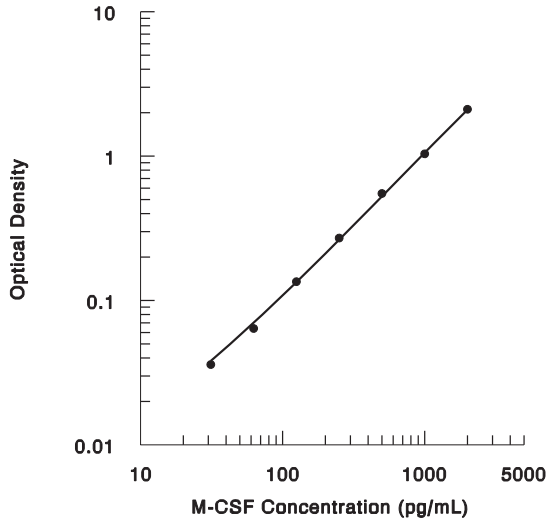
These standard curves are provided for demonstration only. A standard curve should be generated for each set of samples assayed.

Assay Diluent RD1N



(pg/mL)	O.D.	Average	Corrected
0	0.052 0.055	0.054	—
31.2	0.086 0.088	0.087	0.033
62.5	0.125 0.124	0.124	0.070
125	0.207 0.206	0.206	0.152
250	0.343 0.337	0.340	0.286
500	0.657 0.661	0.659	0.605
1000	1.173 1.204	1.188	1.134
2000	2.223 2.234	2.228	2.174

Assay Diluent RD1O



(pg/mL)	O.D.	Average	Corrected
0	0.056 0.054	0.055	—
31.2	0.093 0.088	0.090	0.035
62.5	0.117 0.121	0.119	0.064
125	0.187 0.193	0.190	0.135
250	0.326 0.326	0.326	0.271
500	0.585 0.626	0.606	0.551
1000	1.063 1.120	1.092	1.037
2000	2.196 2.137	2.166	2.111

TECHNICAL HINTS

- When mixing or reconstituting protein solutions, always avoid foaming.
- To avoid cross-contamination, change pipette tips between additions of each standard level, between sample additions, and between reagent additions. Also, use separate reservoirs for each reagent.
- When using an automated plate washer, adding a 30 second soak period following the addition of wash buffer, and/or rotating the plate 180 degrees between wash steps may improve assay precision.
- To ensure accurate results, proper adhesion of plate sealers during incubation steps is necessary.
- Substrate Solution should remain colorless until added to the plate. Keep Substrate Solution protected from light. Substrate Solution should change from colorless to gradations of blue.
- Stop Solution should be added to the plate in the same order as the Substrate Solution. The color developed in the wells will turn from blue to yellow upon addition of the Stop Solution. Wells that are green in color indicate that the Stop Solution has not mixed thoroughly with the Substrate Solution.

PRECISION

Intra-assay Precision (Precision within an assay)
Three samples of known concentration were tested twenty times on one plate to assess intra-assay precision.

Inter-assay Precision (Precision between assays)
Three samples of known concentration were tested in twenty separate assays to assess inter-assay precision.

Serum/Plasma Assay

	Intra-assay Precision			Inter-assay Precision		
Sample	1	2	3	1	2	3
n	20	20	20	20	20	20
Mean (pg/mL)	90.7	227	1134	97.6	232	1194
Standard deviation	4.1	7.7	53.1	5.1	7.3	42.3
CV (%)	4.5	3.4	4.7	5.2	3.1	3.5

Cell Culture Supernate Assay

	Intra-assay Precision			Inter-assay Precision		
Sample	1	2	3	1	2	3
n	20	20	20	20	20	20
Mean (pg/mL)	94.7	230	1190	206	585	1189
Standard deviation	2.8	5.4	19.6	16.2	40.4	57.4
CV (%)	3.0	2.3	1.6	7.9	6.9	4.8

RECOVERY

The recovery of M-CSF spiked to levels throughout the range of the assay in various matrices was evaluated.

Sample Type	Average % Recovery	Range
Cell culture media*	100	84 - 109%
Serum*	95	86 - 102%
EDTA plasma*	99	93 - 106%
Heparin plasma*	98	75 - 113%
Citrate plasma*	98	87 - 108%

*Culture media, serum, and plasma samples were first diluted 2-fold, 5-fold, and 5-fold, respectively into Calibrator Diluent RD6F as directed in the Sample Preparation section.

LINEARITY

To assess the linearity of the assay, five samples (cell culture n=4) were spiked with high concentrations of M-CSF in various matrices and diluted with Calibrator Diluent RD6F to produce samples with values within the dynamic range of the assay.

		Cell culture media*	Serum*	EDTA plasma*	Heparin plasma*	Citrate plasma*
1:2	Average % of Expected	100	101	99	100	100
	Range (%)	98 - 101	99 - 102	97 - 100	94 - 105	96 - 105
1:4	Average % of Expected	97	99	98	99	102
	Range (%)	96 - 99	98 - 101	96 - 100	95 - 106	96 - 109
1:8	Average % of Expected	98	99	95	99	105
	Range (%)	94 - 100	95 - 101	93 - 98	95 - 103	101 - 113
1:16	Average % of Expected	96	99	97	100	106
	Range (%)	94 - 100	96 - 104	94 - 101	93 - 109	92 - 112

*Culture media, serum, and plasma samples were first diluted 2-fold, 5-fold, and 5-fold, respectively into Calibrator Diluent RD6F as directed in the Sample Preparation section.

SENSITIVITY

The minimum detectable dose (MDD) of M-CSF is typically less than 9 pg/mL.

The MDD was determined by adding two standard deviations to the mean optical density value of twenty zero standard replicates and calculating the corresponding concentration.

CALIBRATION

This immunoassay is calibrated against a highly purified *E. coli*-expressed recombinant human M-CSF produced at R&D Systems. The NIBSC/WHO 1st International recombinant human M-CSF Standard 89/512 was evaluated in this kit.

The dose response curve of the NIBSC standard 89/512 parallels the Quantikine standard curve. To convert sample values obtained with the Quantikine Human M-CSF kit to approximate NIBSC International Units, use the equation below:

NIBSC (89/512) approximate value (IU/mL) = 0.060 x Quantikine Human M-CSF value (pg/mL)

SAMPLE VALUES

Fresh Serum/Plasma - Forty samples from apparently healthy volunteers were evaluated for the presence of M-CSF in this assay. No medical histories were available for the donors used in this study.

Sample Type	Mean (pg/mL)	Range (pg/mL)
Serum	670	253 - 1715
EDTA plasma	447	200 - 1193
Citrate plasma	603	288 - 1657
Heparin plasma	377	202 - 962

Cell Culture Supernates - Human peripheral blood mononuclear cells (1 x 10^6 cells/mL) in RPMI supplemented with 10% fetal calf serum, 50 μM β-mercaptoethanol, 2 mM L-glutamine, 100 U/mL penicillin, and 100 μg/mL streptomycin sulfate and were cultured unstimulated or stimulated with 10 μg/mL of PHA. Aliquots of the cell culture supernates were removed and assayed for levels of natural M-CSF.

Conditions	Day 0 (pg/mL)	Day 1 (pg/mL)	Day 3 (pg/mL)	Day 5 (pg/mL)
Unstimulated cells	ND	ND	ND	152
Stimulated cells	ND	850	1447	1432

ND = Non-detectable

SPECIFICITY

This assay recognizes both natural and recombinant human M-CSF. The factors listed below were prepared at 50 ng/mL in Calibrator Diluent RD6F and assayed for cross-reactivity using Assay Diluents RD1N and RD1O. Preparations of the following factors at 50 ng/mL in a mid-range rhM-CSF control were assayed for interference. No significant cross-reactivity or interference was observed.

Recombinant human:
ANG
CNTF
β-ECGF
EGF
Epo
FGF acidic
FGF basic
FGF-4
FGF-5
FGF-6
FGF-7
G-CSF
GM-CSF
sgp130
GROα
GROβ
GROγ
HB-EGF
HGF
IFN-γ
IGF-I
IGF-II
IL-1α
IL-1β
IL-1ra
IL-2
IL-3
IL-3 sRα
IL-3 sRβ
IL-4
IL-5
IL-5 sRα
IL-6
IL-6 sR
IL-7
IL-8
IL-9
IL-10
IL-11
IL-12
IL-13
LAP (TGF-β1)
LIF
MCP-1
MIP-1α
MIP-1β
β-NGF
OSM
PD-ECGF
PDGF-AA
PDGF-AB
PDGF-BB
PTN
RANTES
SCF
SLPI
TGF-α
TGF-β sRII
TGF-β1
TGF-β3
TNF-α
TNF-β
sTNF RI
sTNF RII
VEGF

Recombinant mouse:
GM-CSF
IL-1α
IL-1β
IL-3
IL-4
IL-5
IL-6
IL-7
IL-8
IL-10
IL-13
LIF
MIP-1α
MIP-1β
SCF
TNF-α

Recombinant amphibian:
TGF-β5

Recombinant mouse M-CSF was found to cross-react up to 7%.

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