Quantikine® ELISA

Human M-CSF Immunoassay

Catalog Number DMC00B
SMC00B
PDMC00B

For the quantitative determination of human Macrophage Colony Stimulating Factor (M-CSF) concentrations in cell culture supernates, serum, plasma, saliva, and urine.

This package insert must be read in its entirety before using this product.
For research use only. Not for use in diagnostic procedures.

TABLE OF CONTENTS

SECTION	PAGE
INTRODUCTION	1
PRINCIPLE OF THE ASSAY	2
LIMITATIONS OF THE PROCEDURE	2
TECHNICAL HINTS	2
MATERIALS PROVIDED & STORAGE CONDITIONS	3
OTHER SUPPLIES REQUIRED	4
PRECAUTIONS	4
SAMPLE COLLECTION & STORAGE	4
REAGENT PREPARATION	5
ASSAY PROCEDURE	6
CALCULATION OF RESULTS	7
TYPICAL DATA	7
PRECISION	8
RECOVERY	8
LINEARITY	9
SENSITIVITY	9
CALIBRATION	9
SAMPLE VALUES	10
SPECIFICITY	11
REFERENCES	12
PLATE LAYOUT	13

MANUFACTURED AND DISTRIBUTED BY:

USA & Canada | R&D Systems, Inc.
614 McKinley Place NE, Minneapolis, MN 55413, USA
TEL: (800) 343-7475 (612) 379-2956 FAX: (612) 656-4400
E-MAIL: info@RnDSystems.com

DISTRIBUTED BY:

UK & Europe | R&D Systems Europe, Ltd.
19 Barton Lane, Abingdon Science Park, Abingdon OX14 3NB, UK
TEL: +44 (0)1235 529449 FAX: +44 (0)1235 533420
E-MAIL: info@RnDSystems.co.uk

China | R&D Systems China Co., Ltd.
24A1 Hua Min Empire Plaza, 726 West Yan An Road, Shanghai PRC 200050
TEL: +86 (21) 52380373 FAX: +86 (21) 52371001
E-MAIL: info@RnDSystemsChina.com.cn

INTRODUCTION

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 (1-7). M-CSF is found as isoforms of various sizes. All isoforms contain the N-terminal 150 amino acid (aa) portion that is necessary and sufficient for interaction with the M-CSF receptor but may vary in activity and half-life (7-15). Full length human M-CSF transcripts encode a 522 aa type I transmembrane (TM) protein 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 (11). 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 (12). The region of mature human M-CSF that is common to all forms shares 88%, 86%, 81%, and 74% aa sequence identity with corresponding regions of canine, bovine, mouse, and rat M-CSF, respectively (1, 2). Human M-CSF is active in the mouse, but mouse M-CSF is reported to be species-specific. Sources of M-CSF include fibroblasts, activated macrophages, endometrial secretory epithelium, bone marrow stromal cells, vitamin D-stimulated osteoblasts, and activated endothelial cells (3-8, 16).

The M-CSF receptor (M-CSF R, also called *c-fms*) transduces the pleotropic effects of M-CSF and mediates its endocytosis. Engagement of M-CSF dimers by M-CSF R induces receptor dimerization, followed by phosphorylation at multiple sites (4, 14, 17, 18). M-CSF R is expressed on monocytes and tissue macrophages, and treatment with M-CSF promotes differentiation of macrophages, kidney mesangial cells, liver Kupffer cells, brain microglial cells, bone osteoclasts, fetal trophoblast cells, skin Langerhans cells, intestinal Paneth cells, and blood and lymph node plasmacytoid dendritic cells (3-5, 8, 19). M-CSF R is also expressed on osteoblasts where it downregulates RANKL production, thus allowing M-CSF to limit osteoclast production (20). IL-34 can also engage the M-CSF R, but downstream effects differ (21).

M-CSF is essential for macrophage-related functions such as bone resorption, vascular development, and innate immunity. M-CSF-deficient (op/op) mice are deficient in macrophages and are osteopetrotic due to insufficient differentiation of bone-resorbing osteoclasts (3, 6-8, 20, 22). They also show failure of teeth eruption, infertility, and defects in development of nervous, vascular, and lymphatic systems (4, 7, 16, 22). M-CSF regulates the release of cytokines and other inflammatory modulators from macrophages, and stimulates chemotaxis and pinocytosis (4, 5, 7). Circulating M-CSF increases during pregnancy and supports implantation and growth of the decidua and placenta (3, 7, 16). M-CSF expression can also be increased during infection or in inflammatory disorders such as inflammatory bowel disease (3, 5, 6). Both M-CSF and its receptor can be expressed by a number of cancers, allowing M-CSF to act as an autocrine growth factor for cancer cells. Macrophages can also be recruited to tumor tissues, supplying M-CSF as a paracrine growth factor (23). On the other hand, M-CSF can have anti-cancer effects by priming and enhancing macrophage killing of tumor cells and microorganisms (3). It is thought that expression of M-CSF R on cancer cells facilitates metastasis to the bone by chemotaxis toward osteoblast-produced M-CSF and by promoting osteolysis (3, 24).

The Quantikine Human M-CSF Immunoassay is a 4.5 hour solid phase ELISA designed to measure human M-CSF in cell culture supernates, serum, plasma, saliva, and urine. It contains CHO cell-expressed recombinant human M-CSF and has been shown to accurately quantitate the recombinant factor. Results obtained using natural human M-CSF showed linear curves that were parallel to the standard curves obtained using the Quantikine kit standards. These results indicate that this kit can be used to determine relative mass values for naturally occurring human M-CSF.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for human 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 human 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, dilute the samples with the appropriate 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.
- Variations in sample collection, processing, and storage may cause sample value differences.
- This assay is designed to eliminate interference by other factors present in biological samples. Until all factors have been tested in the Quantikine Immunoassay, the possibility of interference cannot be excluded.

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.
- To ensure accurate results, proper adhesion of plate sealers during incubation steps is necessary.
- 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.
- 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.

MATERIALS PROVIDED & STORAGE CONDITIONS

Store the unopened kit at 2-8 °C. Do not use past kit expiration date.

PART	PART #	CATALOG # DMC00B	CATALOG # SDMC00B	DESCRIPTION	STORAGE OF OPENED/ RECONSTITUTED MATERIAL
Human M-CSF Microplate	893868	1 plate	6 plates	96 well polystyrene microplate (12 strips of 8 wells) coated with a monoclonal antibody specific for human M-CSF.	Return unused wells to the foil pouch containing the desiccant pack. Reseal along entire edge of the zip-seal. May be stored for up to 1 month at 2-8 °C.*
Human M-CSF Conjugate	893869	1 vial	6 vials	21 mL/vial of polyclonal antibody specific for human M-CSF conjugated to horseradish peroxidase with preservatives.	May be stored for up to 1 month at 2-8 °C.*
Human M-CSF Standard	893870	1 vial	6 vials	50 ng/vial of recombinant human M-CSF in a buffered protein base with preservatives; lyophilized.	
Assay Diluent RD1-56	895102	1 vial	6 vials	17 mL/vial of a buffered protein solution with preservatives.	
Calibrator Diluent RD5-18	895335	1 vial	6 vials	21 mL/vial of a buffered protein base with preservatives. *For cell culture supernate/saliva/urine samples.*	
Calibrator Diluent RD6P	895118	1 vial	6 vials	21 mL/vial of animal serum with preservatives. *For serum/plasma samples.*	
Wash Buffer Concentrate	895003	1 vial	6 vials	21 mL/vial of a 25-fold concentrated solution of buffered surfactant with preservative. *May turn yellow over time.*	
Color Reagent A	895000	1 vial	6 vials	12 mL/vial of stabilized hydrogen peroxide.	
Color Reagent B	895001	1 vial	6 vials	12 mL/vial of stabilized chromogen (tetramethylbenzidine).	
Stop Solution	895032	1 vial	6 vials	6 mL/vial of 2 N sulfuric acid.	
Plate Sealers	N/A	4 strips	24 strips	Adhesive strips.	

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

DMC00B contains sufficient materials to run an ELISA on one 96 well plate.
SMC00B (SixPak) contains sufficient materials to run ELISAs on six 96 well plates.

This kit is also available in a PharmPak (R&D Systems, Catalog # PDMC00B). 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.

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 of standards.
- 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.

The Stop Solution provided with this kit is an acid solution.

Some components in this kit contain ProClin® which may cause an allergic skin reaction. Avoid breathing mist.

Color Reagent B may cause skin, eye, and respiratory irritation. Avoid breathing fumes.

Wear protective gloves, clothing, eye, and face protection. Wash hands thoroughly after handling. Please refer to the MSDS on our website prior to use.

SAMPLE COLLECTION & STORAGE

The sample collection and storage conditions listed below are intended as general guidelines. Sample stability has not been evaluated.

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

Serum - Use a serum separator tube (SST) and allow samples to clot for 30 minutes at room temperature before centrifugation for 15 minutes at 1000 x g. Remove serum and assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

Plasma - Collect plasma using EDTA or heparin as an anticoagulant. Centrifuge for 15 minutes at 1000 x g within 30 minutes of collection. Assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

Note: *Citrate plasma is not recommended for use in this assay.*

Saliva - Collect saliva in a tube and centrifuge for 5 minutes at 10,000 x g. Collect the aqueous layer, and assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

Note: *Saliva values are decreased when a Salivette® or other collection device is used. When stored at 2-8 °C, saliva sample values increase over time.*

Urine - Aseptically collect the first urine of the day (mid-stream), voided directly into a sterile container. Centrifuge to remove particulate matter, assay immediately or aliquot and store at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

REAGENT PREPARATION

Bring all reagents to room temperature before use.

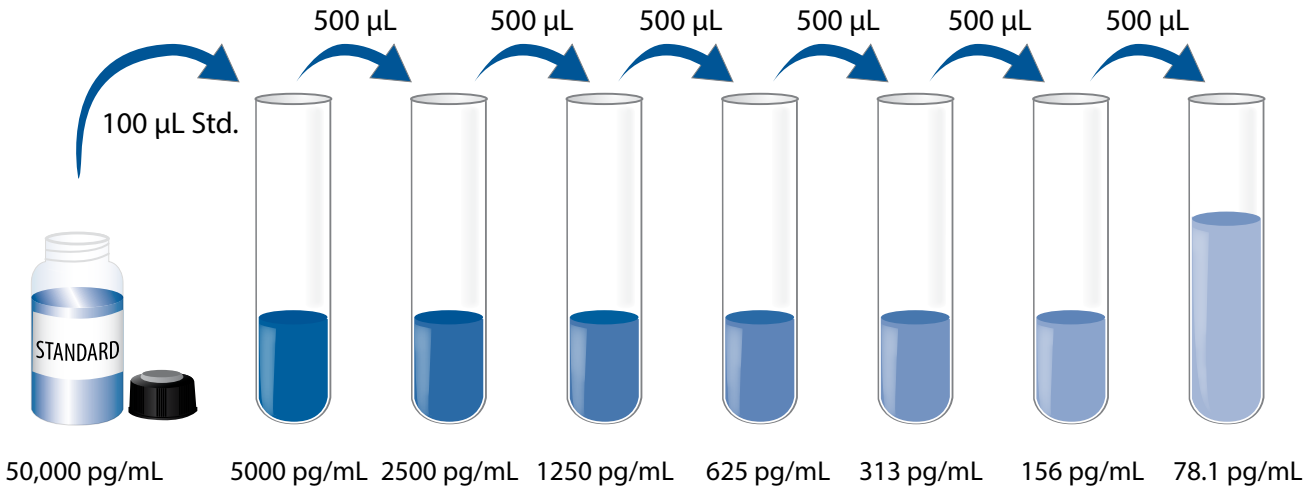
Note: *High concentrations of M-CSF are found in saliva. It is recommended that a face mask and gloves be used to protect kit reagents from contamination.*

Wash Buffer - If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. Add 20 mL of Wash Buffer Concentrate to 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.

Human M-CSF Standard - Reconstitute the Human M-CSF Standard with 1.0 mL of deionized or distilled water. This reconstitution produces a stock solution of 50,000 pg/mL. Allow the standard to sit for a minimum of 15 minutes with gentle agitation prior to making dilutions.

Pipette 900 µL of Calibrator Diluent RD5-18 (*for cell culture supernate/saliva/urine samples*) or Calibrator Diluent RD6-P (*for serum/plasma samples*) into the 5000 pg/mL tube. Pipette 500 µL of the appropriate Calibrator Diluent into the remaining tubes. Use the stock solution to produce a dilution series (below). Mix each tube thoroughly before the next transfer. The 5000 pg/mL standard serves as the high standard. The appropriate Calibrator Diluent 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 standards, samples, and controls be assayed in duplicate.

Note: *High concentrations of M-CSF are found in saliva. It is recommended that a face mask and gloves be used to protect kit reagents from contamination.*

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. Add 100 µL of Assay Diluent RD1-56 to each well.
4. Add 100 µL of Standard, control, or sample per well. Cover with the adhesive strip provided. Incubate for 2 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 Human M-CSF Conjugate to each well. Cover with a new adhesive strip. Incubate for 2 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.

CALCULATION OF RESULTS

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

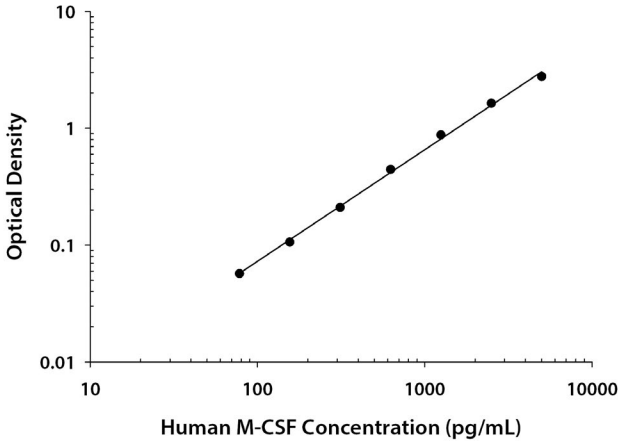
Create a standard curve by reducing the data using computer software capable of generating a log/log curve-fit. As an alternative, construct a standard curve by plotting the mean absorbance for each standard on the y-axis against the concentration on the x-axis and draw a best fit curve through the points on a log/log graph. The data may be linearized by plotting the log of the human M-CSF concentrations versus the log of the O.D. on a linear scale, and the best fit line can be determined by regression analysis.

If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.

TYPICAL DATA

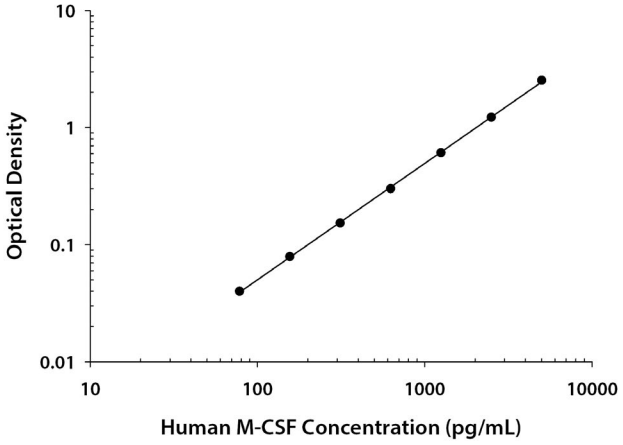
These standard curves are provided for demonstration only. A standard curve should be generated for each set of samples assayed.

CELL CULTURE SUPERNATES/SALIVA/URINE ASSAY



(pg/mL)	O.D.	Average	Corrected
0	0.017 0.017	0.017	—
78.1	0.071 0.076	0.074	0.057
156	0.115 0.131	0.123	0.106
313	0.216 0.237	0.227	0.210
625	0.447 0.475	0.461	0.444
1250	0.868 0.917	0.893	0.876
2500	1.606 1.689	1.648	1.631
5000	2.610 2.958	2.784	2.767

SERUM/PLASMA ASSAY



(pg/mL)	O.D.	Average	Corrected
0	0.036 0.037	0.037	—
78.1	0.071 0.074	0.073	0.036
156	0.106 0.115	0.111	0.074
313	0.177 0.187	0.182	0.146
625	0.341 0.356	0.349	0.312
1250	0.658 0.666	0.662	0.626
2500	1.279 1.348	1.314	1.277
5000	2.467 2.535	2.501	2.465

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 forty separate assays to assess inter-assay precision. Assays were performed by at least three technicians using two lots of components.

CELL CULTURE SUPERNATE/SALIVA/URINE ASSAY

	Intra-Assay Precision			Inter-Assay Precision		
Sample	1	2	3	1	2	3
n	20	20	20	40	40	40
Mean (pg/mL)	248	751	1503	280	827	1620
Standard deviation	19.4	16.1	49.0	30.2	76.0	87.7
CV (%)	7.8	2.1	3.3	10.8	9.2	5.4

SERUM/PLASMA ASSAY

	Intra-Assay Precision			Inter-Assay Precision		
Sample	1	2	3	1	2	3
n	20	20	20	40	40	40
Mean (pg/mL)	357	951	1767	343	1019	1956
Standard deviation	17.4	24.9	49.4	43.4	84.1	88.8
CV (%)	4.9	2.6	2.8	12.7	8.3	4.5

RECOVERY

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

Sample Type	Average % Recovery	Range
Cell culture media (n=4)	99	90-107%
Serum (n=4)	101	96-106%
EDTA plasma (n=4)	100	93-106%
Heparin plasma n=4)	99	93-106%

LINEARITY

To assess linearity of the assay, samples containing and/or spiked with high concentrations of human M-CSF were diluted with the appropriate Calibrator Diluent to produce samples with values within the dynamic range of the assay.

		Cell culture supernates (n=4)	Serum (n=4)	EDTA plasma (n=4)	Heparin plasma (n=4)	Saliva (n=4)	Urine (n=4)
1:2	Average % of Expected	104	94	94	97	97	96
	Range (%)	97-109	90-98	91-99	90-102	92-107	92-103
1:4	Average % of Expected	103	97	101	102	93	94
	Range (%)	93-113	93-99	92-106	92-109	84-105	90-103
1:8	Average % of Expected	105	101	105	102	96	92
	Range (%)	91-115	98-108	100-112	95-109	88-103	84-106
1:16	Average % of Expected	101	104	106	98	88	98
	Range (%)	85-109	99-111	100-119	92-104	83-93	82-114

SENSITIVITY

Eighty assays were evaluated and the minimum detectable dose (MDD) of M-CSF ranged from 1.74-47.3 pg/mL. The mean MDD was 11.2 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 223 amino acid CHO cell-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) = 0.042 x Quantikine Human M-CSF value (pg/mL)

SAMPLE VALUES

Serum/Plasma/Saliva/Urine - Samples from apparently healthy volunteers were evaluated for the presence of human 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)	Standard Devation (pg/mL)
Serum (n=36)	296	180-474	80.7
EDTA plasma (n=36)	207	108-374	65.2
Heparin plasma (n=36)	266	134-434	79.0
Saliva (n=7)	1312	312-2994	1100
Urine (n=14)	1413	263-4466	1336

Cell Culture Supernates:
Human peripheral blood leukocytes were cultured in DMEM supplemented with 5% bovine calf serum, 5 µM β-mercaptoethanol, 2 mM L-glutamine, 100 U/mL penicillin, and 100 µg/mL streptomycin sulfate. Cells were cultured unstimulated or stimulated with 10 µg/mL of PHA for 1 and 5 days. Aliquots of the cell culture supernates were removed and assayed for levels of human M-CSF.

Condition	Day 1 (pg/mL)	Day 5 (pg/mL)
Unstimulated cells	ND	156
Stimulated cells	205	933

ND=Non-detectable

A549 human lung carcinoma cells were cultured in MEM and supplemented with 5% bovine calf serum until confluent. An aliquot of the cell culture supernate was removed, assayed for human M-CSF, and measured 5304 pg/mL.

U2OS human osteosarcoma cells were cultured in McCoy's 5a and supplemented with 15% bovine calf serum until confluent. An aliquot of the cell culture supernate was removed, assayed for human M-CSF, and measured 156 pg/mL.

MG-63 human osteosarcoma cells were cultured in MEM supplemented with 10% bovine calf serum and NEAA until confluent. An aliquot of the cell culture supernate was removed, assayed for human M-CSF, and measured 12,292 pg/mL.

SPECIFICITY

This assay recognizes natural and recombinant human M-CSF.

The factors listed below were prepared at 50 ng/mL in Calibrator Diluent and assayed for cross-reactivity. Preparations of the following factors at 50 ng/mL in a mid-range recombinant human M-CSF control were assayed for interference. No significant cross-reactivity or interference was observed.

Recombinant human:

β-ECGF
EGF
FGF-4
FGF-5
FGF-6
FGF-9
FGF-10
FGF-18
FGF acidic
FGF basic
Flt-3/Flk-2 Ligand
Flt-4
G-CSF
GM-CSF
HB-EGF
HGF
IGF-I
IGF-II
KGF/FGF-7
M-CSF R
MSP
β-NGF
NRG1-α/HRG1-α
PD-ECGF
PDGF-AA
PDGF-AB
PDGF-BB
PDGF-CC
PDGF-DD
PDGF Rα
PDGF Rβ
PlGF
$VEGF_{121}$
$VEGF_{165}$
VEGF/PlGF
VEGF-D

Recombinant mouse:

FGF-8b
FGF-8c
Flt-3/Flk-2 Ligand
G-CSF
GM-CSF
M-CSF R
$VEGF_{120}$
$VEGF_{164}$
PDGF-CC
PDGF Rα
PDGF Rβ
PlGF-2

Recombinant rat:

GM-CSF
β-NGF
PDGF-AA
PDGF-AB
PDGF-BB

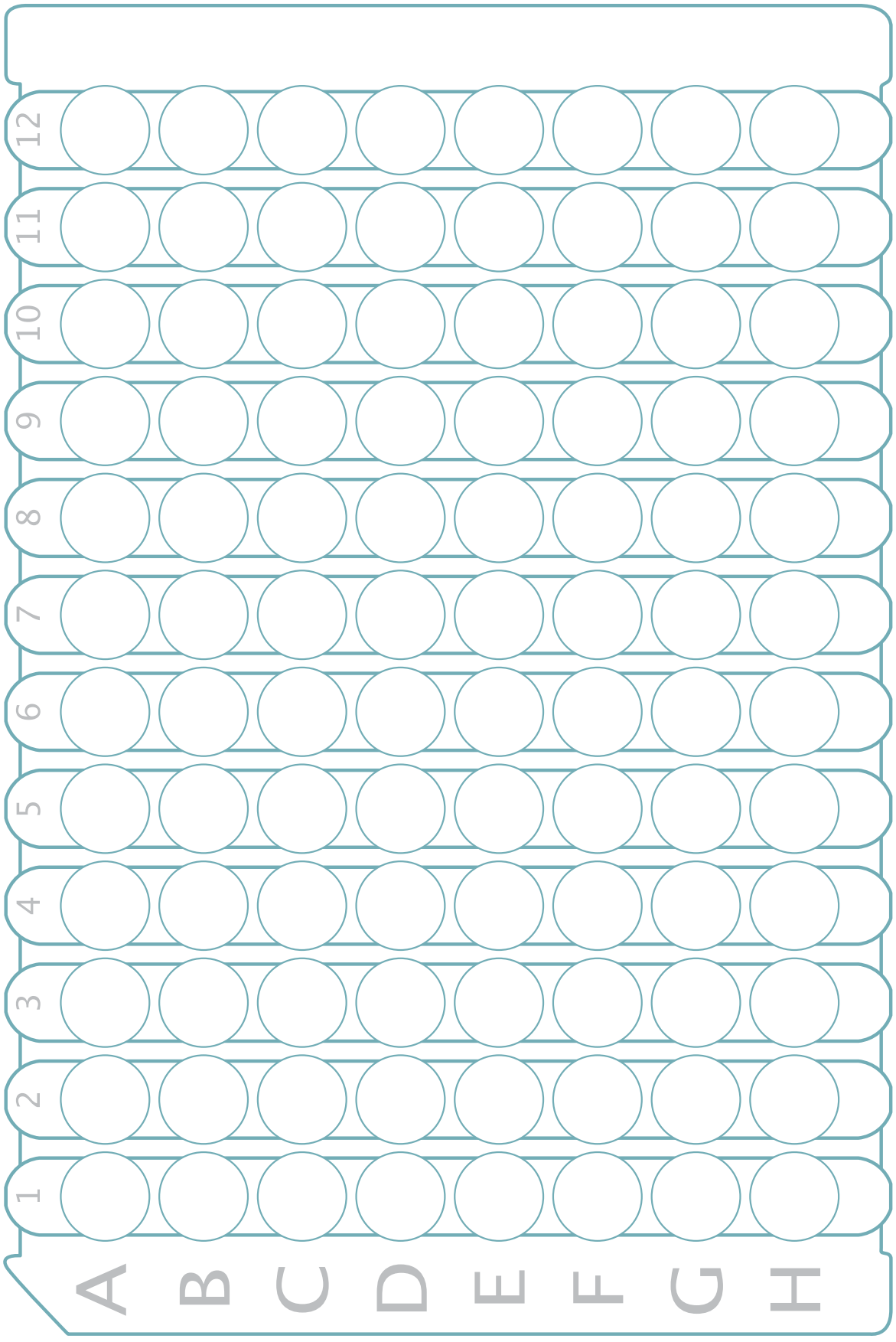
Recombinant mouse M-CSF cross-reacts approximately 0.18% in this assay.

REFERENCES

1. Kawasaki, E.S. *et al.* (1985) Science **230**:291.
2. Wong, G.G. *et al.* (1987) Science **235**:1504.
3. Douglass, T.G. *et al.* (2008) Int. Immunopharmacol. **8**:1354.
4. Pixley, F.J. and E.R. Stanley (2004) Trends Cell Biol. **14**:628.
5. Chitu, V. and E.R. Stanley (2006) Curr. Opin. Immunol. **18**:39.
6. Gow, D.J. *et al.* (2010) J. Leukoc. Biol. **88**:475.
7. Fixe, P. and V. Praloran (1997) Eur. Cytokine Netw. **8**:125.
8. Ryan, G.R. *et al.* (2001) Blood **98**:74.
9. Nandi, S. *et al.* (2006) Blood **107**:786.
10. Suzu, S. *et al.* (1998) Biochem. Biophys. Res. Commun. **245**:120.
11. Suzu, S. *et al.* (1992) J. Biol. Chem. **267**:16812.
12. Rettenmier, C.W. and M.F. Roussel (1988) Mol. Cell Biol. **8**:5026.
13. Manos, M.M. (1988) Mol. Cell. Biol. **8**:5035.
14. Koths, K. (1997) Mol. Reprod. Dev. **46**:31.
15. Jang, M-H. *et al.* (2006) J. Immunol. **177**:4055.
16. Makrigiannakis, A. *et al.* (2006) Trends Endocrinol. Metab. **17**:178.
17. Takeshita, S. *et al.* (2007) J. Biol. Chem. **282**:18980.
18. Faccio, R. *et al.* (2007) J. Biol. Chem. **282**:18991.
19. Huynh, D. *et al.* (2009) Gastroenterology **137**:136.
20. Wittrant, Y. *et al.* (2009) Endocrinology **150**:4977.
21. Chihara, T. *et al.* (2010) Cell Death Differ. **17**:1917.
22. Kubota, Y. *et al.* (2009) J. Exp. Med. **206**:1089.
23. Wang, L. *et al.* (2008) Cancer Res. **68**:5639.
24. Yagiz, K. and S.R. Rittling (2009) Exp Cell Res. **315**:2442.

PLATE LAYOUT

Use this plate layout to record standards and samples assayed.



NOTES