Quantikine® ELISA

Mouse G-CSF Immunoassay

Catalog Number MCS00

For the quantitative determination of mouse Granulocyte-Colony Stimulating Factor (G-CSF) concentrations in cell culture supernates and serum.

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

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

Mouse granulocyte-colony stimulating factor (G-CSF) is a 24-25 kDa monomeric glycoprotein that regulates the proliferation, differentiation and activation of hematopoietic cells (1, 2). Mouse G-CSF cDNA encodes a 208 amino acid (aa) precursor protein with a 30 aa signal sequence that is proteolytically cleaved to form a 178 aa O-glycosylated mature protein containing two intrachain disulfide bridges (2, 3, 4). In humans, two distinct cDNA clones, encoding a 204 aa form and a minor alternatively spliced 207 aa form of G-CSF precursors, have been isolated (5, 6). Mouse and human G-CSF are 76% identical at the aa sequence level and the two proteins show species cross-reactivity (2, 4, 7). G-CSF is produced primarily by monocytes and macrophages upon activation by endotoxin, TNF-α or IL-1 (8). Other cell types, including fibroblasts, endothelial cells, astrocytes and bone marrow stroma cells, can also secrete G-CSF after activation (8). In addition, various tumor cells express G-CSF constitutively (8, 9).

Mouse G-CSF receptor (G-CSF R) is a 120 kDa, type I transmembrane glycoprotein that belongs to the hematopoietin receptor superfamily (10-12). The mature protein is 812 amino acids in length and contains a 601 aa extracellular region, a 24 aa transmembrane segment, and a 187 aa cytoplasmic domain (10, 11). The extracellular region contains multiple modules, including an N-terminal Ig-like domain, a cytokine receptor homology domain, and three fibronectin type III domains (12-14). Based on crystallographic study, G-CSF receptor forms a complex with the ligand in a 2:2 ratio (13, 14). Mouse and human G-CSF receptor share 63% aa sequence identity (7, 8). Cells known to express G-CSF R include monocytes and neutrophils (15), megakaryocytes and platelets (16), $CD34^+$ $CD33^+$ and $CD34^+$ $CD38^+$ hematopoietic progenitors (17), trophoblasts (18), endothelial cells (19) and various tumor cell types.

G-CSF is an important regulator for granulopoiesis *in vivo* (8). It has been demonstrated that G-CSF can support the growth of multi-lineage hematopoietic progenitor cells without influencing their commitment to the myeloid lineage and mobilize hematopoietic progenitor cells from the bone marrow into the bloodstream (8, 21-25). On mature neutrophils, G-CSF may regulate neutrophil survival by controlling their rate of apoptosis (24, 26). G-CSF has also been shown to enhance the functional capacity of mature neutrophils (20, 26, 27). As a consequence of its effects on hematopoietic progenitor cells, G-CSF has been shown to enhance monocytopoiesis in the presence M-CSF (28, 29). Within the peripheral blood stem cell population mobilized with G-CSF, selective increases in the number of T helper 2-inducing dendritic cells are found (30).

The Quantikine® Mouse G-CSF Immunoassay is a 4.5 hour solid phase ELISA designed to measure mouse G-CSF in cell culture supernates and serum. It contains *E. coli*-expressed recombinant mouse G-CSF and antibodies raised against the recombinant factor. This immunoassay has been shown to quantitate the recombinant mouse G-CSF accurately. Results obtained using natural mouse G-CSF showed dose-response curves that were parallel to the standard curves obtained using the recombinant Quantikine® kit standards. These results indicate that this kit can be used to determine relative mass values for natural mouse G-CSF.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. A polyclonal antibody specific for mouse G-CSF has been pre-coated onto a microplate. Standards, control, and samples are pipetted into the wells and any G-CSF present is bound by the immobilized antibody. After washing away any unbound substances, an enzyme-linked polyclonal antibody specific for mouse G-CSF is added to the wells. Following a wash to remove any unbound antibody-enzyme reagent, a substrate solution is added to the wells. The enzyme reaction yields a blue product that turns yellow when the Stop Solution is added. The intensity of the color measured is in proportion to the amount of G-CSF bound in the initial step. The sample values are then read off the standard curve.

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 calibrator diluent and repeat the assay.
- Any variation in 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.
- 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.

MATERIALS PROVIDED & STORAGE CONDITIONS

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

PART	PART #	DESCRIPTION	STORAGE OF OPENED/ RECONSTITUTED MATERIAL
Mouse G-CSF Microplate	890825	96 well polystyrene microplate (12 strips of 8 wells) coated with a polyclonal antibody specific for mouse G-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.*
Mouse G-CSF Standard	890827	Recombinant mouse G-CSF in a buffered protein base with preservatives; lyophilized. *Refer to the vial label for reconstitution volume.*	Aliquot and store for up to 1 month at ≤ -20 °C.*
Mouse G-CSF Control	890393	Recombinant mouse G-CSF in a buffered protein base with preservatives; lyophilized. The assay value of the control should be within the range specified on the label.	
Mouse G-CSF Conjugate	890826	12 mL of a polyclonal antibody specific for mouse G-CSF conjugated to horseradish peroxidase with preservatives.	May be stored for up to 1 month at 2-8 °C.*
Assay Diluent RD1-54	895321	12 mL of animal serum with preservatives.	
Calibrator Diluent RD5-16	895302	21 mL of diluted animal serum with preservatives.	
Wash Buffer Concentrate	895003	21 mL of a 25-fold concentrated solution of buffered surfactant with preservative. *May turn yellow over time.*	
Color Reagent A	895000	12 mL of stabilized hydrogen peroxide.	
Color Reagent B	895001	12 mL of stabilized chromogen (tetramethylbenzidine).	
Stop Solution	895174	23 mL of diluted hydrochloric acid.	
Plate Sealers	N/A	4 adhesive strips.	

* 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 of standards and samples.

PRECAUTIONS

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

Some components in this kit contain a preservative 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. 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. Assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

Serum - Allow blood samples to clot for 2 hours at room temperature before centrifuging for 20 minutes at 2000 x g. Remove serum and assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

Note: *Grossly hemolyzed or lipemic samples may not be suitable for use in this assay.*

SAMPLE PREPARATION

Cell culture supernate samples require a 2-fold dilution prior to assay. A suggested 2-fold dilution is 75 µL of sample + 75 µL of Calibrator Diluent RD5-16.

REAGENT PREPARATION

Bring all reagents to room temperature before use.

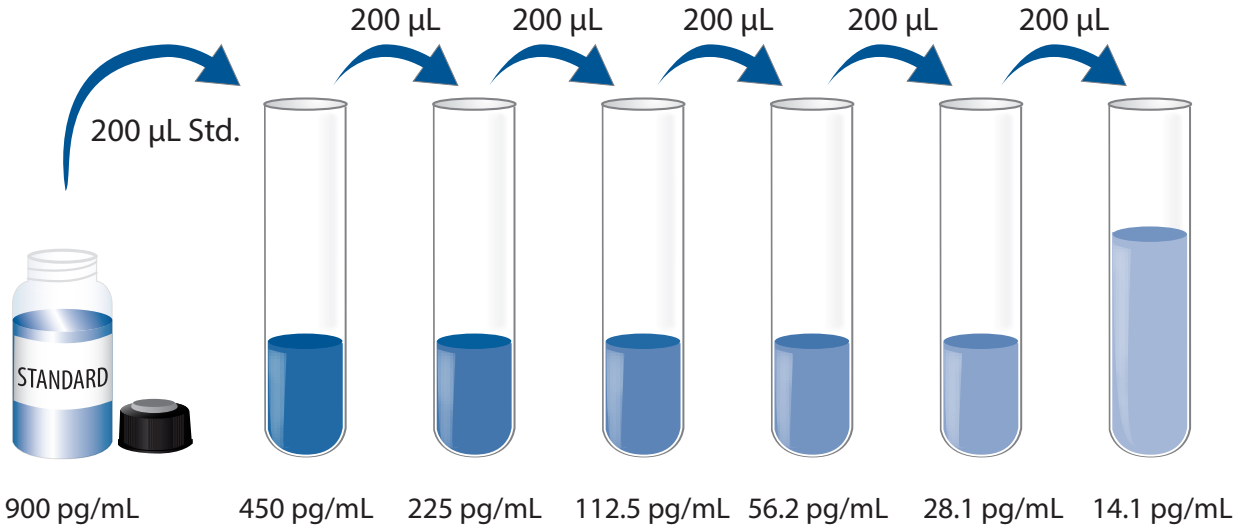
Mouse G-CSF Control - Reconstitute the control with 1.0 mL of deionized or distilled water. Mix thoroughly. Assay the control undiluted.

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. 100 µL of the resultant mixture is required per well.

Mouse G-CSF Standard - **Refer to the vial label for reconstitution volume.** Reconstitute the Mouse G-CSF Standard with Calibrator Diluent RD5-16. Do not substitute other diluents. This reconstitution produces a stock solution of 900 pg/mL. Allow the standard to sit for a minimum of 5 minutes with gentle mixing prior to making dilutions.

Pipette 200 µL of Calibrator Diluent RD5-16 into each tube. Use the stock solution to produce a dilution series (below). Mix each tube thoroughly before the next transfer. The undiluted Mouse G-CSF Standard (900 pg/mL) serves as the high standard. Calibrator Diluent RD5-16 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, control, and samples be assayed in duplicate.

1. Prepare reagents, standard dilutions, and control as directed in the previous section.
2. Remove excess microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, and reseal.
3. Add 50 µL of Assay Diluent RD1-54 to each well.
4. Add 50 µL of standard, control, or sample* per well. Mix by gently tapping the plate frame for 1 minute. Cover with the adhesive strip provided. Incubate for 2 hours at room temperature.
5. Aspirate each well and wash, repeating the process four times for a total of five 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 100 µL of Mouse G-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 100 µL of Substrate Solution to each well. Incubate for 30 minutes at room temperature. **Protect from light.**
9. Add 100 µL of Stop Solution to each well. 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.

*Samples may require dilution. See Sample Preparation section.

CALCULATION OF RESULTS

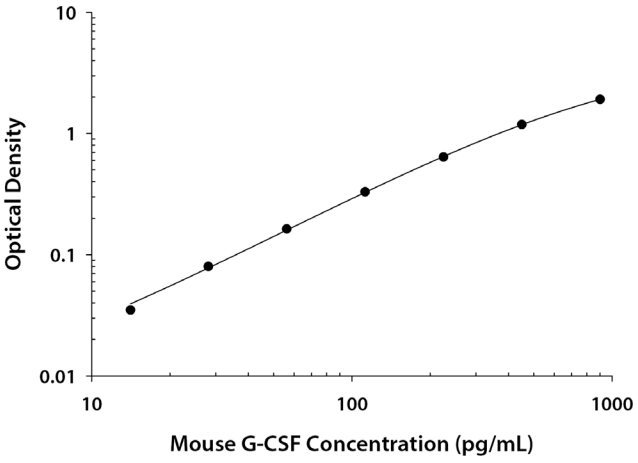
Average the duplicate readings for each standard, control, and sample and subtract the average zero standard optical density (O.D.).

Create a standard curve by reducing the data using computer software capable of generating a 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 mouse G-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.

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

TYPICAL DATA

This standard curve is provided for demonstration only. A standard curve should be generated for each set of samples assayed.



(pg/mL)	O.D.	Average	Corrected
0	0.040 0.041	0.040	—
14.1	0.073 0.077	0.075	0.035
28.1	0.118 0.121	0.120	0.080
56.2	0.197 0.209	0.203	0.163
112.5	0.366 0.375	0.370	0.330
225	0.671 0.687	0.679	0.639
450	1.207 1.245	1.226	1.186
900	1.960 1.943	1.952	1.912

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. Assays were performed by at least three technicians using two lots of components.

	Intra-Assay Precision			Inter-Assay Precision		
Sample	1	2	3	1	2	3
n	20	20	20	20	20	20
Mean (pg/mL)	64.4	196	697	64.8	195	620
Standard deviation	2.3	8.8	43	4.1	15.5	50.6
CV (%)	3.6	4.5	6.2	6.3	7.9	8.2

RECOVERY

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

Sample Type	Average % Recovery	Range
Cell culture supernates* (n=7)	92	82-110%
Serum (n=5)	96	80-119%

*Samples were diluted prior to assay.

LINEARITY

To assess the linearity of the assay, five or more samples containing and/or spiked with various concentrations of mouse G-CSF in each matrix were diluted with calibrator diluent and then assayed. Results from typical sample dilutions are shown.

Sample	Dilution	Observed (pg/mL)	Expected (pg/mL)	$\frac{\text{Observed}}{\text{Expected}} \times 100$
Cell culture supernates*	Neat	256	———	———
	1:2	122	128	95%
	1:4	63	64	98%
	1:8	32	32	100%
	1:16	17	16	106%
Serum	Spiked	349	———	———
	1:2	186	174	107%
	1:4	89	87	102%
	1:8	43	44	98%
	1:16	19	22	86%

*Samples were diluted prior to assay.

SENSITIVITY

The minimum detectable dose (MDD) of mouse G-CSF is typically less than 5 pg/mL.

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

CALIBRATION

This immunoassay is calibrated against a highly purified *E. coli*-expressed recombinant mouse G-CSF produced at R&D Systems®.

SAMPLE VALUES

Serum - Samples were evaluated for the presence of mouse G-CSF in this assay.

Sample Type	Mean (pg/mL)	Range (pg/mL)	Standard Deviation (pg/mL)
Serum (n=40)	193	36.7-1967	416

Cell Culture Supernates - Mouse splenocytes were cultured in RPMI supplemented with 10% fetal bovine serum and 5 µg/mL LPS. An aliquot of the cell culture supernate was removed, assayed for mouse G-CSF, and measured 167 pg/mL.

SPECIFICITY

This assay recognizes natural and recombinant mouse G-CSF.

The factors listed below were prepared at 50 ng/mL in calibrator diluent and assayed for cross-reactivity. Preparations of the following factors prepared at 50 ng/mL in a mid-range mouse G-CSF control were assayed for interference. No significant cross-reactivity or interference was observed.

Recombinant mouse:

C10
Eotaxin
Flt-3 Ligand
GM-CSF
IFN-γ
IL-1α
IL-1β
IL-1ra
IL-2
IL-3
IL-4
IL-5
IL-6
IL-7
IL-9
IL-10
IL-10 R
IL-12 p40
IL-12 p70
IL-13
IL-17
IL-18 R
JE/MCP-1
KC
Leptin
LIF
MARC
MCP-5
M-CSF
MIP-1α
MIP-2
OSM
PlGF-2
RANTES
SCF
TNF-α
TNF RI
TNF RII
Tpo
VEGF
VEGF R1/Flt-1

Recombinant human:

G-CSF
GM-CSF
VEGF
VEGF/PlGF

REFERENCES

1. Metcalf, D. (1986) Blood **67**:257.
2. Tsuchiya, M. *et al.* (1986) Proc. Natl. Acad. Sci. USA **83**:7633.
3. Nagata, S. *et al.* (1986) Nature **319**:415.
4. Souza, L.M. *et al.* (1986) Science **232**:61.
5. Tsuchiya, M. *et al.* (1987) Eur. J. Biochem. **165**:7.
6. Nagata, S. *et al.* (1986) EMBO J. **5**:575.
7. Fukunaga, R. *et al.* (1990) Proc. Natl. Acad. Sci. USA **87**:8702.
8. Murakami, H. and S. Nagata (1998) in *The Cytokine Handbook*, 3rd edition, A. Thomson, editor, Academic Press, New York, 671.
9. Usuda, H. *et al.* (1997) Pathol. Intl. **47**:866.
10. Fukunaga, R. *et al.* (1990) Cell **61**:341.
11. Fukunaga, R. *et al.* (1990) J. Biol. Chem. **265**:14008.
12. Larsen, A. *et al.* (1990) J. Exp. Med. **172**:1559.
13. Layton, J.E. *et al.* (1999) J. Biol. Chem. **274**:17445.
14. Aritomi, M. *et al.* (1999) Nature **401**:713.
15. Boneberg, E-M. *et al.* (2000) Blood **95**:270.
16. Kanaji, T. *et al.* (1995) Blood **85**:3359.
17. Ebihara, Y. *et al.* (2000) Br. J. Hematol. **109**:153.
18. Uzumaki, H. *et al.* (1989) Proc. Natl. Acad. Sci. USA **86**:9323.
19. Bussolino, F. *et al.* (1989) Nature **337**:471.
20. Siekermann, K. *et al.* (1997) Leukemia **11**:466.
21. Semerad, C.L. *et al.* (1999) Immunity **11**:153.
22. Liu, F. *et al.* (1997) Blood **90**:2583.
23. Yang, F-C. *et al.* (1998) Blood **92**:4632.
24. Liu, F. *et al.* (1996) Immunity **5**:491.
25. Liu, F. *et al.* (2000) Blood **95**:3025.
26. Leavey, P.J. *et al.* (1998) Blood **92**:4366.
27. Pajkrt, D. *et al.* (1997) Blood **90**:1415.
28. Gilmore, G. *et al.* (1995) Exp. Hematol. **23**:1319.
29. Ohsaka, A. (1996) Exp. Hematol. **24**:767.
30. Arpinati, M. *et al.* (2000) Blood **95**:2484.