Quantikine® HS ELISA

Human GM-CSF Immunoassay

Catalog Number HSGM0

For the quantitative determination of human Granulocyte Macrophage Colony Stimulating Factor (GM-CSF) concentrations in serum, plasma, and urine.

Note: The standard reconstitution method has changed. Please read this package insert in its entirety before using this product.

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

Granulocyte macrophage colony stimulating factor (GM-CSF) is a pleiotropic cytokine with multiple effects on hematopoietic cells (1-4). It mobilizes $CD34^+$ progenitor cells into the periphery and stimulates their proliferation, survival and differentiation into neutrophils, monocytes/macrophages, eosinophils, and myeloid dendritic cells (4-8). On these terminally-differentiated myeloid cells, GM-CSF is also needed for inducing their effector functions (7-10). In addition, GM-CSF has been shown to stimulate the proliferation and differentiation of the erythroid and megakaryoctye progenitor cells (4). GM-CSF is produced by a number of different cell types, including keratinocytes (11), mature and immature NK cells (12), type II alveolar cells (13), endothelial cells (14), monocytes (14,15), bone-marrow mesenchymal stem cells (18), $CD4^+$ and $CD8^+$ T cells (17), megakaryocytes (18), B cells (19), eosinophils (20), chondrocytes (21) and fibroblasts (22).

Human GM-CSF cDNA encodes a 144 amino acid (aa) residue precursor protein with a 17 aa putative signal peptide and a 127 aa mature protien (23-25). Natural GM-CSF is a monomer that contains both N- and O-linked glycosylation (28). Mature human GM-CSF shares approximately 55%, 63% and 68% aa sequence homology with mouse (27), rat (28) and canine (29) GM-CSF, respectively. Human GM-CSF is not biologically active on mouse cells (23), but was reported to have some activity on canine cells (29).

GM-CSF exerts its activity through binding to a high affinity receptor complex consisting of two membrane glycoproteins: a GM-CSF-specific receptor α chain that binds GM-CSF with low-affinity, and a β chain that does not bind GM-CSF directly but is required for conferring high-affinity binding to the receptor complex (30-32). The β chain is also a component of the IL-3 and IL-5 receptor complexes. The stoichiometry for a functional GM-CSF signaling receptor is reported to be a 2:2:2 complex of GM-CSF, receptor α subunit and β subunit (30-33). Alternatively, spliced variants for both the α and the β receptor subunits are known (32, 35). The presence of the spliced variants in the heteromeric receptor complex can regulate the functions of the complex (36).

The Quantikine® HS Human GM-CSF Immunoassay is a 6.5 hour solid phase ELISA designed to measure human GM-CSF in serum, plasma, and urine. It contains *E. coli*-expressed recombinant human GM-CSF and antibodies raised against the recombinant factor. It has been shown to accurately quantitate the recombinant factor. Results obtained using natural GM-CSF showed linear curves that were parallel to the standard curves obtained using the Quantikine® HS kit standards. These results indicate that this kit can be used to determine relative mass values for human GM-CSF.

PRINCIPLE OF THE ASSAY

DUE TO THE HIGH SENSITIVITY OF THIS KIT, PLEASE NOTE THE FOLLOWING PRECAUTIONS.

- **Alkaline phosphatase is detectable in saliva.** Take precautionary measures (*e.g. wear a mask and gloves*) to protect reagents during preparation and use and while running the assay (reagent preparation through the addition of Stop Solution).
- Inorganic phosphate is a strong competitive inhibitor of alkaline phosphatase; avoid the use of PBS based wash buffers and other sources of inorganic phosphate contamination.
- Use separate pipettes and glassware for dispensing all reagents to avoid cross-contamination.
- Careful washing of the microplate is essential to minimize nonspecific binding of the conjugate.

This assay employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for human GM-CSF has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any GM-CSF present is bound by the immobilized antibody. After washing away any unbound substances, an enzyme-linked monoclonal antibody specific for human GM-CSF is added to the wells. Following a wash to remove any unbound antibody-enzyme reagent, a substrate solution is added to the wells. After an incubation period, an amplifier solution is added to the wells and color develops in proportion to the amount of GM-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.
- It is important that the calibrator diluent selected for the standard curve be consistent with the samples being assayed.
- 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

- To ensure accurate results, bring liquids to room temperature and mix to homogeneity prior to pipetting or aliquoting.
- When mixing 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.
- Long incubations are known to cause edge effects in immunoassays. Incubating in a moist environment has been shown to minimize these effects.
- Neither the addition of the Substrate Solution nor the Stop Solution will result in a color change.
- Addition of the Amplifier Solution will result in a color change (colorless to shades of maroon).
- Amplifier Solution and Stop Solution should be added to the plate in the same order as the Substrate Solution.

PRECAUTIONS

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.

Alkaline phosphatase is detectable in saliva. Take precautionary measures (*e.g. wear a mask and gloves*) to protect reagents during preparation and use and while running the assay (reagent preparation through the addition of Stop Solution).

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.

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

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
Human GM-CSF HS Microplate	890184	96 well polystyrene microplate (12 strips of 8 wells) coated with a monoclonal antibody specific for human GM-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 GM-CSF HS Standard	890186	Recombinant human GM-CSF in a buffered protein base with preservatives; lyophilized. *Refer to the vial label for reconstitution volume.*	Store for up to 1 month at ≤ -20 °C in a manual defrost freezer.* Avoid repeated freeze-thaw cycles.
Human GM-CSF HS Conjugate	890185	21 mL of a monoclonal antibody specific for human GM-CSF conjugated to alkaline phosphatase with preservatives.	Store for up to 1 month at 2-8 °C.*
Assay Diluent HD1R	895062	11 mL of a buffered protein base with preservatives. *For urine samples.*	
Assay Diluent HD1-3	895213	11 mL of a buffered protein base with preservatives. *May be slightly cloudy. For serum/plasma samples.*	
Calibrator Diluent RD5C	895046	21 mL of a buffered protein base with preservatives. *For urine samples.*	
Calibrator Diluent RD6-11	895489	21 mL of a buffered protein base with preservatives. *For serum/plasma samples.*	
Wash Buffer Concentrate	895188	100 mL of a 10-fold concentrated solution of buffered surfactant with preservative. *May turn yellow over time.*	
Stop Solution	895032	6 mL of 2 N sulfuric acid.	
Substrate	895884	Lyophilized NADPH with stabilizers.	**Store in an upright position** for up to 1 month at ≤ -20 °C in a manual defrost freezer.* Avoid repeated freeze-thaw cycles.
Substrate Diluent	895885	7 mL of buffered solution with stabilizers and preservative.	
Amplifier	895886	Lyophilized amplifier enzymes with stabilizers.	
Amplifier Diluent	895887	7 mL of buffered solution containing INT-violet with stabilizer and preservative.	
Plate Sealers	N/A	8 Adhesive strips.	

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

OTHER SUPPLIES REQUIRED

- Microplate reader capable of measuring absorbance at 490 nm, with the correction wavelength set at 650 nm or 690 nm.
- Pipettes and pipette tips.
- Deionized or distilled water.
- Squirt bottle, manifold dispenser or autowasher.
- 1000 mL graduated cylinder.
- Test tubes for dilution of standards.
- Human GM-CSF Controls (optional; R&D Systems®, Catalog # QC01-1).

SAMPLE COLLECTION & STORAGE

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

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.*

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: *Alkaline phosphatase is detectable in saliva.* *Take precautionary measures (e.g. wear a mask and gloves) to protect reagents during preparation and use and while running the assay (reagent preparation through the addition of Stop Solution).*

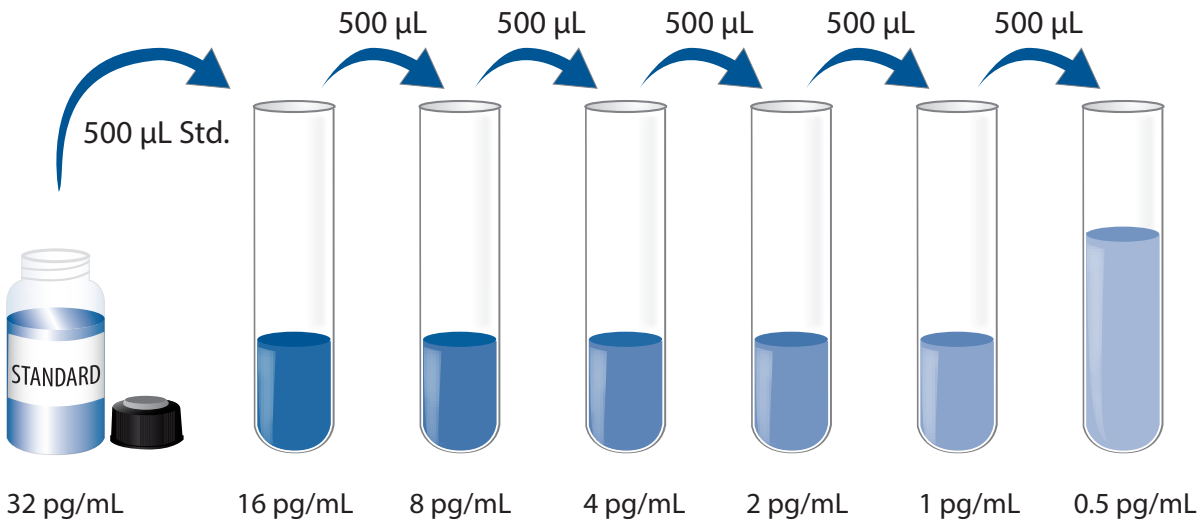
Wash Buffer - If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. Add 100 mL of Wash Buffer Concentrate to deionized or distilled water to prepare 1000 mL of Wash Buffer.

Substrate Solution - Reconstitute the lyophilized Substrate in 6.0 mL of Substrate Diluent at least 10 minutes before use. **Re-stopper and re-cap the vial**, and mix thoroughly. Avoid contamination.

Amplifier Solution - Reconstitute the lyophilized Amplifier in 6.0 mL of Amplifier Diluent at least 10 minutes before use. **Re-stopper and re-cap the vial**, and mix thoroughly. Avoid contamination.

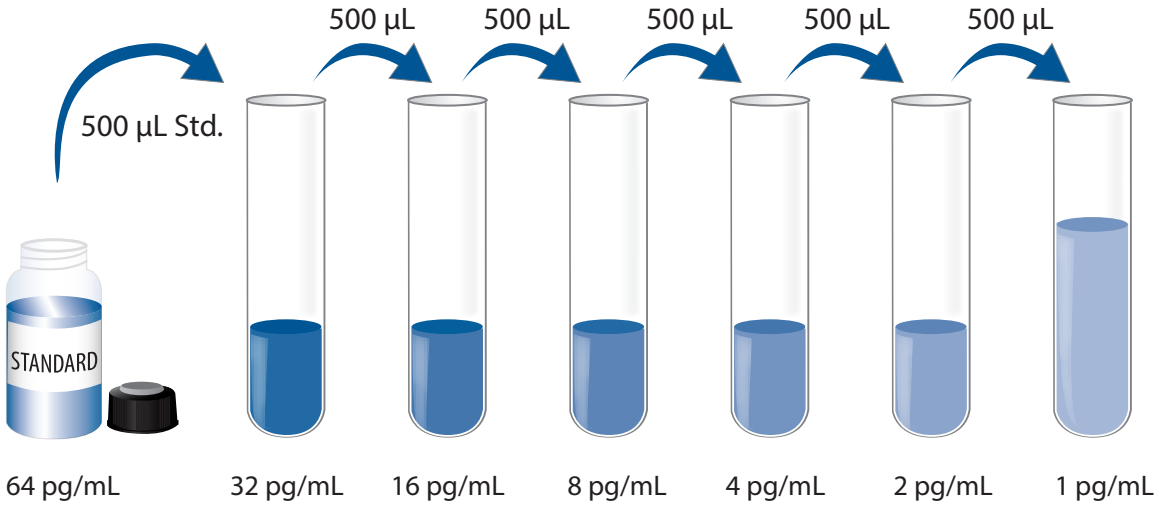
Human GM-CSF HS Standard - **Refer to the vial label for reconstitution volume.** Reconstitute the Human GM-CSF HS Standard with Calibrator Diluent RD5C (*for urine samples*) or 2.5 mL of Calibrator Diluent RD6-11 (*for serum/plasma samples*). This reconstitution produces a stock solution of 32 pg/mL and 64 pg/mL, respectively. Allow the standard to sit for a minimum of 15 minutes with gentle agitation prior to making dilutions.

For Urine Samples: Pipette 500 µL of Calibrator Diluent RD5C into each tube. Use the stock solution to produce a dilution series (below). Mix each tube thoroughly before the next transfer. The undiluted Human GM-CSF HS Standard (32 pg/mL) serves as the high standard. Calibrator Diluent RD5C serves as the zero standard (0 pg/mL).



REAGENT PREPARATION *CONTINUED*

For Serum/Plasma Samples: Pipette 500 µL of Calibrator Diluent RD6-11 into each tube. Use the stock solution to produce a dilution series (below). Mix each tube thoroughly before the next transfer. The undiluted Human GM-CSF HS Standard (64 pg/mL) serves as the high standard. Calibrator Diluent RD6-11 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: *Alkaline phosphatase is detectable in saliva.* *Take precautionary measures to protect reagents (e.g. wear a mask and gloves).*

1. Prepare all reagents and working standards 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 Assay Diluent to each well.
   For Urine Samples: Add 50 µL of Assay Diluent HD1R.
   For Serum/Plasma Samples: Add 100 µL of Assay Diluent HD1-3.
4. Add the appropriate amount of standard, sample, or control per well.
   For Urine Samples: Add 200 µL of Standard, sample, or control per well.
   For Serum/Plasma Samples: Add 150 µL of Standard, sample, or control per well.

   Cover with the adhesive strip provided. Incubate for 3 hours at room temperature.
5. Wash
   a. Remove liquid from the wells by aspirating or inverting the plate and decanting the contents.
   b. Remove excess liquid by grasping the plate firmly and smartly rapping the inverted plate on a clean paper towel at least 5 times.
   c. Fill each well with 400 µL of Wash Buffer using a squirt bottle, manifold dispenser, or autowasher.
   d. Remove liquid from the wells by aspirating or inverting the plate and decanting the contents.
   e. Repeat steps b, c, and d 5 times for a total of 6 washes. After the last wash, smartly rap the inverted plate on a clean paper towel at least 10 times to remove excess Wash Buffer.
6. Add 200 µL of Human GM-CSF HS Conjugate to each well. Cover with a new adhesive strip. Incubate for 2 hours at room temperature.
7. Repeat the wash as in step 5.
8. Add 50 µL of Substrate Solution to each well. Cover with a new adhesive strip. Incubate for 1 hour at room temperature. **Do not wash the plate.**
9. Add 50 µL of Amplifier Solution to each well. Cover with a new adhesive strip. Incubate for 30 minutes at room temperature. Addition of Amplifier Solution initiates color development.
10. Add 50 µL of Stop Solution to each well. Addition of Stop Solution does not affect the color in the wells.
11. Determine the optical density of each well within 30 minutes, using a microplate reader set to 490 nm. If wavelength correction is available, set to 650 nm or 690 nm. If wavelength correction is not available, subtract readings at 650 nm or 690 nm from the readings at 490 nm. This subtraction will correct for optical imperfections in the plate. Readings made directly at 490 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.).

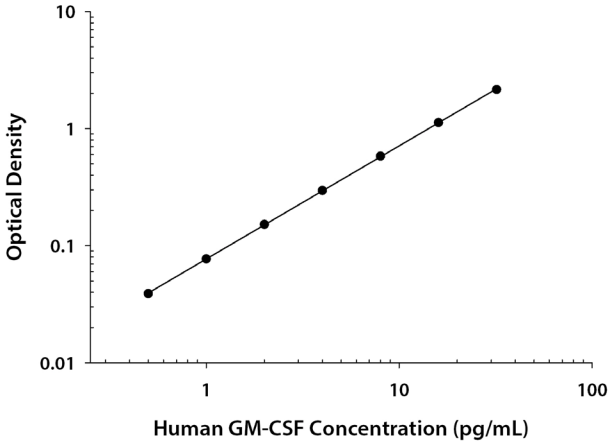
Create a standard curve by reducing the data using computer software capable of generating a log/log curve-fit. As an alternative, construct a standard curve by plotting the mean absorbance for each standard on the y-axis against the concentration on the x-axis and draw a best fit curve through the points on a log/log graph. The data may be linearized by plotting the log of the human GM-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

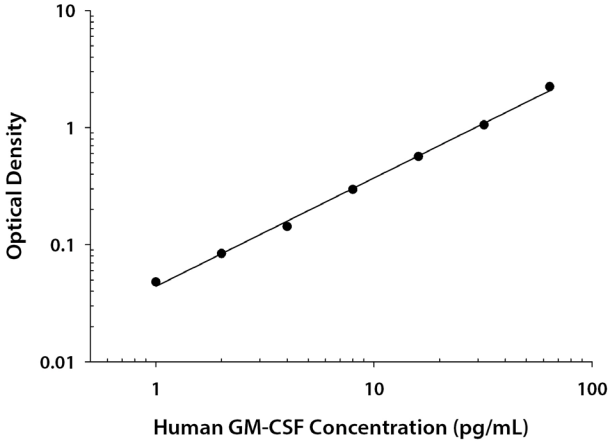
These standard curves are provided for demonstration only. A standard curve should be generated for each set of samples assayed.

URINE ASSAY



(pg/mL)	O.D.	Average	Corrected
0	0.098 0.096	0.097	—
0.5	0.135 0.136	0.136	0.039
1	0.172 0.176	0.174	0.077
2	0.249 0.249	0.249	0.152
4	0.399 0.387	0.393	0.296
8	0.678 0.679	0.678	0.581
16	1.233 1.206	1.220	1.123
32	2.212 2.296	2.254	2.157

SERUM/PLASMA ASSAY



(pg/mL)	O.D.	Average	Corrected
0	0.116 0.118	0.117	—
1	0.164 0.165	0.165	0.048
2	0.201 0.201	0.201	0.084
4	0.259 0.260	0.260	0.143
8	0.407 0.419	0413	0.296
16	0.676 0.693	0.684	0.567
32	1.164 1.182	1.173	1.056
64	2.266 2.437	2.352	2.235

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

URINE ASSAY

	Intra-Assay Precision			Inter-Assay Precision		
Sample	**1**	**2**	**3**	**1**	**2**	**3**
n	20	20	20	20	20	20
Mean (pg/mL)	2.69	7.05	24.8	3.77	14.3	27.4
Standard deviation	0.14	0.33	0.73	0.37	1.17	1.85
CV (%)	5.2	4.7	2.9	9.8	8.2	6.8

SERUM/PLASMA ASSAY

	Intra-Assay Precision			Inter-Assay Precision		
Sample	**1**	**2**	**3**	**1**	**2**	**3**
n	20	20	20	18	18	18
Mean (pg/mL)	3.68	22.0	44.6	2.80	16.2	33.2
Standard deviation	0.35	1.07	1.76	0.66	2.47	4.60
CV (%)	9.5	4.9	3.9	23.6	15.2	13.9

RECOVERY

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

Sample Type	Average % Recovery	Range
Serum (n=4)	109	94-128%
EDTA plasma (n=4)	114	88-140%
Heparin plasma (n=4)	109	91-122%
Urine (n=5)	102	95-116%

LINEARITY

To assess the linearity of the assay, samples spiked with high concentrations of human GM-CSF were serially diluted with calibrator diluent to produce samples with values within the dynamic range of the assay.

		Serum (n=4)	EDTA plasma (n=4)	Heparin plasma (n=4)	Urine (n=10)
1:2	Average % of Expected	90	92	100	105
	Range (%)	81-96	88-98	96-104	100-112
1:4	Average % of Expected	86	91	99	97
	Range (%)	78-90	86-94	93-102	90-108
1:8	Average % of Expected	83	88	98	100
	Range (%)	71-90	81-94	89-104	85-110
1:16	Average % of Expected	91	95	102	103
	Range (%)	83-99	92-101	89-113	86-117

SENSITIVITY

The minimum detectable dose (MDD) of GM-CSF is typically less than 0.26 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 highly purified *E. coli*-expressed recombinant human GM-CSF produced at R&D Systems®.

The human GM-CSF NIBSC/WHO International Standard 88/646 (recombinant, human-sequence protein expressed in *E. coli*) was evaluated in this kit. The dose response curve in this International Standard parallels the Quantikine® HS standard curve.

To convert sample values obtained with the Quantikine® HS Human GM-CSF kit to approximate NIBSC/WHO 88/646 International units, use the equation below.

NIBSC/WHO (88/646) approximate value (IU/mL) = 0.0083 x Quantikine® HS Human GM-CSF value (pg/mL).

SAMPLE VALUES

Serum/Plasma/Urine - Samples from apparently healthy volunteers were evaluated for the presence of human GM-CSF in this assay. No medical histories were available for the donors used in this study.

Sample Type	Mean of Detectable (pg/mL)	% Detectable	Range (pg/mL)
Serum (n=37)	1.72	14	ND-2.19
EDTA plasma (n=37)	1.56	14	ND-2.20
Heparin plasma (n=37)	1.07	3	ND-1.07
Urine (n=37)	0.80	53	ND-1.46

ND-Non-detectable

SPECIFICITY

This assay recognizes natural and recombinant human GM-CSF.

The factors listed below were prepared at 10 ng/mL in calibrator diluent and assayed for cross-reactivity. Preparations of the following factors at 10 ng/mL in a mid-range recombinant human GM-CSF control were assayed for interference. No significant cross-reactivity or interference was observed.

Recombinant human:

G-CSF
GM-CSF Rα
GM-CSF Rβ
M-CSF

Recombinant mouse:

GM-CSF

REFERENCES

1. Lieschke, G.J. and A.B. Burgess (1992) N. Engl. J. Med. **327**:28.
2. Armitage, J.O. (1998) Blood **92**:4491.
3. Baldwin, G.C. (1992) Dev. Biol. **151**:352.
4. Hill, A.D.K. *et al.* (1995) J. Leukoc. Biol. **58**:634.
5. Metcalf, D. (1986) Blood **67**:257.
6. Gazitt, Y. (2002) Curr. Opin. Hematol. **9**:190.
7. Gilliet, M. *et al.* (2002) J. Exp. Med. **195**:953.
8. Basak, S.K. *et al.* (2002) Blood **99**:2869.
9. Jablonska, E. *et al.* (2002) Melanoma Res. **12**:123.
10. Metcalf, D. *et al.* (1986) Blood **67**:37.
11. Mann, A. *et al.* (2001) J. Invest. Dermatol. **117**:1382.
12. Loza, M.J. *et al.* (2002) Blood **99**:1273.
13. Christensen, P.J. *et al.* (1995) Am. J. Respir. Cell Mol. Biol. **13**:426.
14. Takahashi, M. *et al.* (1996) Circulation **93**:1185.
15. Lee, M-T. *et al.* (1990) J. Leukoc. Biol. **47**:275.
16. Majumdar, M.K. *et al.* (2000) J. Hematother. Stem Cell Res. **9**:841.
17. Till, S. *et al.* (1995) Eur. J. Immunol. **25**:2727.
18. Wickenhauser, C. *et al.* (1995) Blood **85**:685.
19. Harris, R.J. *et al.* (2000) J. Immunol. **164**:3887.
20. Levi-Schaffer, F. *et al.* (1995) Blood **85**:2579.
21. Yonezawa, I. *et al.* (1996) Biochem. Biophys. Res. Commun. **219**:261.
22. Saada, A. *et al.* (1996) J. Cell. Biol. **133**:159.
23. Cantrell, M.A. *et al.* (1985) Proc. Natl. Acad. Sci. USA **82**:6250.
24. Wong, G.C. *et al.* (1985) Science **228**:810.
25. Lee, F. *et al.* (1985) Proc. Natl. Acad. Sci. USA **82**:4360.
26. Schrimsher, J.L. *et al.* (1987) Biochem. J. **247**:195.
27. Gough, N.M. *et al.* (1985) EMBO J. **4**:645.
28. Smith, L.R. *et al.* (1994) Immunogenetics **39**:80.
29. Nash, R.A. *et al.* (1991) Blood **78**:930.
30. Guthridge, M.A. *et al.* (1998) Stem Cells **16**:301.
31. Gearing, D.P. *et al.* (1989) EMBO J. **8**:3667.
32. Hayashida, K. *et al.* (1990) Proc. Natl. Acad. Sci. USA **87**:9655.
33. D'Andrea, R.J. and T.J. Gonda (2000) Exp. Hematol. **28**:231.
34. Hu, X. *et al.* (1994) Biochim. Biophys. Acta **1223**:306.
35. Gale, R.E. *et al.* (1998) Blood **91**:54.
36. Wagner, K. *et. al.* (2001) Blood **98**:2689.

PLATE LAYOUT

Use this plate layout to record standards and samples assayed.