Quantikine® ELISA

Human GM-CSF Immunoassay

Catalog Number DGM00
SGM00
PDGM00

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

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.

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INTRODUCTION

Granulocyte-Macrophage Colony Stimulating Factor (GM-CSF), also known as CSF-2, is a pleiotrophic 30 kDa member of the Common beta Chain (βc) cytokine family that also includes IL-3 and IL-5. GM-CSF adopts an α-helical configuration with two intrachain disulfide bonds. It is secreted by a wide variety of activated immune, mesenchymal, and epithelial cell types and circulates as a variably glycosylated monomer (1, 2). It is upregulated in multiple cell types during inflammation including encephalitogenic T cells (3-5), allergen exposed lung endothelial cells (6), and IgE activated mast cells (7). Mature human GM-CSF shares 54% and 63% amino acid sequence identity with mouse and rat GM-CSF, respectively (8).

The high-affinity receptor for GM-CSF is composed of a 50 kDa ligand binding alpha subunit (GM-CSF Rα) (9) and a 120 kDa signal transducing βc (10, 11). The stoichiometry for the functional GM-CSF receptor is reported to be a 2:2:2 complex of GM-CSF, GM-CSF Rα, and βc (12, 13). Notably, the βc subunit is shared by the receptor complexes for IL-3 and IL-5, and IL-5 may signal through GM-CSF Rα and βc (13). GM-CSF may additionally utilize Syndecan-2 as a co-receptor (14).

A number of functions have been attributed to GM-CSF. It induces monocyte, neutrophil, and eosinophil production from $CD34^+$ stem cell precursors (15, 16). It can act in concert with IL-4 or Flt-3 Ligand to induce the development and maintenance of myeloid and dermal dendritic cells (16-20). It also acts as a neutrophil and dendritic cell chemoattractant (6, 21). GM-CSF promotes Th1 and Th17 cell mediated autoimmune inflammation as well as the inflammatory activation of dendritic cells, microglia, alveolar macrophages, and eosinophils (3-5, 22-26). In addition, it cooperates with G-CSF in promoting tumor cell proliferation and invasion (27).

The Quantikine® Human GM-CSF Immunoassay is a 3.5-4.5 hour solid phase ELISA designed to measure human GM-CSF levels in cell culture supernates, serum, and plasma. It contains *E. coli*-expressed recombinant human GM-CSF and antibodies raised against the recombinant factor. It has been shown to quantitate recombinant human GM-CSF accurately. Results obtained using natural human GM-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 human GM-CSF.

PRINCIPLE OF THE ASSAY

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 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

- 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 # DGM00	CATALOG # SGM00	DESCRIPTION	STORAGE OF OPENED/ RECONSTITUTED MATERIAL
Human GM-CSF Microplate	890027	1 plate	6 plates	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 Standard	890029	1 vial	6 vials	Recombinant human GM-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 in a manual defrost freezer.* Avoid repeated freeze-thaw cycles.
Human GM-CSF Conjugate	890479	1 vial	6 vials	21 mL/vial of a monoclonal antibody specific for human GM-CSF conjugated to horseradish peroxidase with preservatives.	May be stored for up to 1 month at 2-8 °C.*
Assay Diluent RD1-6	895158	1 vial	6 vials	11 mL/vial of a buffered protein base with preservatives. *May contain a precipitate. Mix well before and during use.*	
Calibrator Diluent RD5-5	895485	1 vial	6 vials	21 mL/vial of a buffered protein base with preservatives. *For cell culture supernate 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.

DGM00 contains sufficient materials to run an ELISA on one 96 well plate.
SGM00 (SixPak) contains sufficient materials to run ELISAs on six 96 well plates.

This kit is also available in a PharmPak (R&D Systems®, Catalog # PDGM00). PharmPaks contain sufficient materials to run ELISAs on 50 microplates. Specific vial counts of each component may vary. 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 GM-CSF Controls (optional; R&D Systems®, Catalog # QC01-1).

PRECAUTIONS

Some components in 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.

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 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, heparin, or citrate 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.

REAGENT PREPARATION

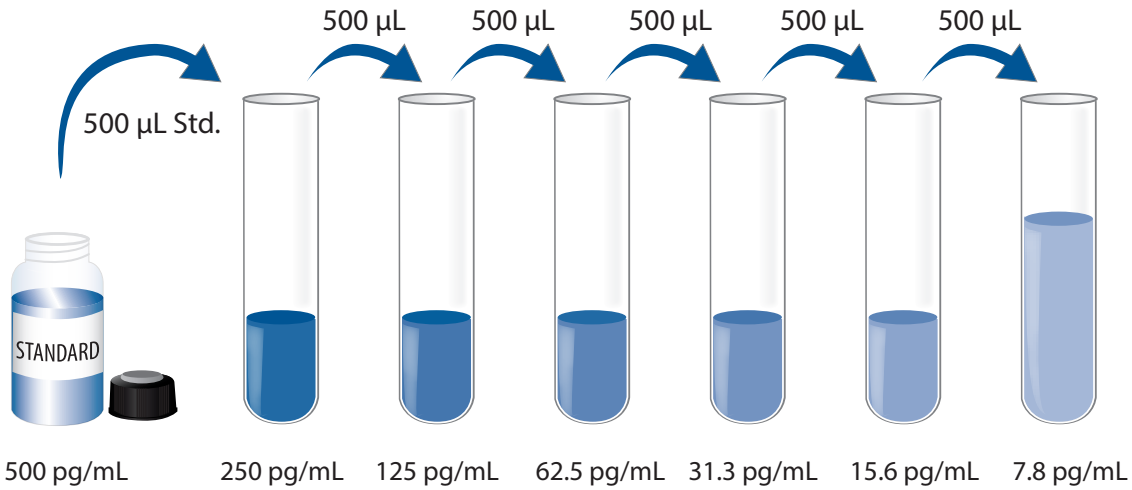
Bring all reagents to room temperature before use.

Wash Buffer - If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. 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 GM-CSF Standard - **Refer to the vial label for reconstitution volume.** Reconstitute the Human GM-CSF Standard with Calibrator Diluent RD5-5 (*for cell culture supernate samples*) or Calibrator Diluent RD6P (*for serum/plasma samples*). This reconstitution produces a stock solution of 500 pg/mL. Allow the standard to sit for a minimum of 15 minutes with gentle agitation prior to making dilutions.

Pipette 500 µL of the appropriate calibrator diluent 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 Standard (500 pg/mL) 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.

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 100 µL of Assay Diluent RD1-6 to each well. *Assay Diluent RD1-6 may contain a precipitate. Mix well before and during use.*
4. Add 100 µL of standard, control, or sample per well. **Ensure reagent addition is uninterrupted and completed within 15 minutes.** Cover with the adhesive strip provided. Incubate for 2 hours at room temperature.
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 GM-CSF Conjugate to each well. Cover with a new adhesive strip.
   For Cell Culture Supernate Samples: Incubate for 1 hour at room temperature.
   For Serum/Plasma Samples: 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 20 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.).

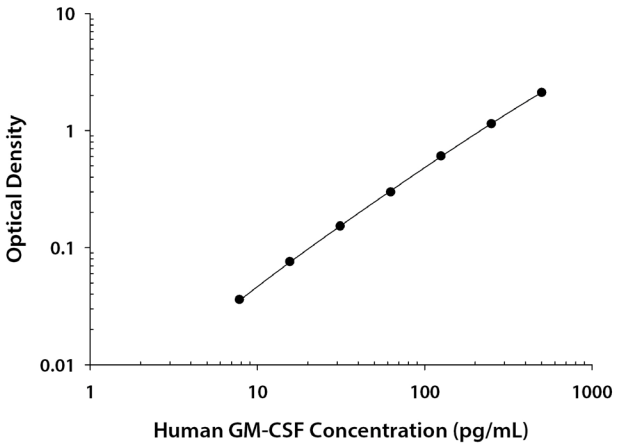
Create a standard curve by reducing the data using computer software capable of generating a four parameter logistic (4-PL) curve fit. As an alternative, construct a standard curve by plotting the mean absorbance for each standard on the y-axis against the concentration on the x-axis and draw a best fit curve through the points on the graph. The data may be linearized by plotting the log of the human GM-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

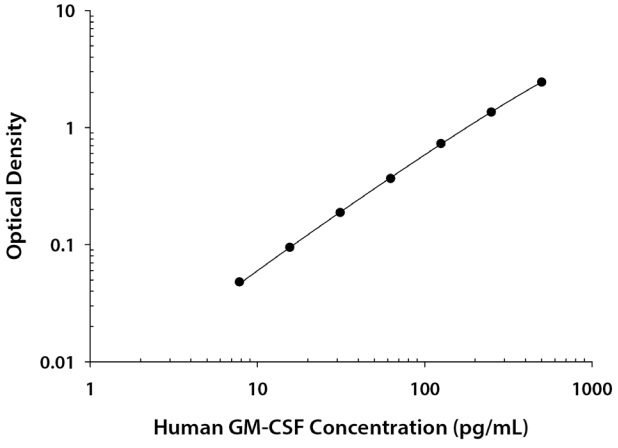
These standard curves are provided for demonstration only. A standard curve should be generated for each set of samples assayed.

CELL CULTURE SUPERNATE ASSAY



(pg/mL)	O.D.	Average	Corrected
0	0.017 0.018	0.018	—
7.8	0.057 0.052	0.054	0.036
15.6	0.092 0.095	0.094	0.076
31.3	0.173 0.169	0.171	0.153
62.5	0.320 0.314	0.317	0.299
125	0.636 0.611	0.624	0.606
250	1.186 1.136	1.161	1.143
500	2.147 2.126	2.136	2.118

SERUM/PLASMA ASSAY



(pg/mL)	O.D.	Average	Corrected
0	0.014 0.017	0.016	—
7.8	0.064 0.065	0.064	0.048
15.6	0.113 0.109	0.111	0.095
31.3	0.204 0.204	0.204	0.188
62.5	0.389 0.379	0.384	0.368
125	0.749 0.742	0.746	0.730
250	1.368 1.377	1.372	1.356
500	2.442 2.485	2.464	2.448

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 ASSAY

	Intra-Assay Precision			Inter-Assay Precision		
Sample	**1**	**2**	**3**	**1**	**2**	**3**
n	20	20	20	40	40	40
Mean (pg/mL)	46.8	131	269	45.1	133	266
Standard deviation	1.1	3.6	7.3	2.4	7.0	11.5
CV (%)	2.4	2.7	2.7	5.3	5.3	4.3

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)	54.7	156	321	53.1	157	317
Standard deviation	1.1	3.2	4.8	3.3	9.1	15.4
CV (%)	2.0	2.1	1.5	6.2	5.8	4.9

RECOVERY

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

Sample Type	Average % Recovery	Range
Cell culture media (n=5)	97	85-104%
Serum (n=5)	98	93-103%
EDTA plasma (n=5)	100	94-105%
Heparin plasma (n=5)	98	92-104%
Citrate plasma (n=5)	101	92-106%

SENSITIVITY

The minimum detectable dose (MDD) of human GM-CSF is typically less than 3 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.

LINEARITY

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

		Cell culture media (n=5)	Serum (n=5)	EDTA plasma (n=5)	Heparin plasma (n=5)	Citrate plasma (n=5)
1:2	Average % of Expected	104	100	99	102	99
	Range (%)	99-110	98-104	97-101	101-104	98-104
1:4	Average % of Expected	105	101	99	101	97
	Range (%)	96-113	99-103	96-102	99-104	96-102
1:8	Average % of Expected	109	98	100	100	97
	Range (%)	104-112	97-100	98-103	99-104	95-101
1:16	Average % of Expected	———	98	101	98	97
	Range (%)	———	96-100	98-104	97-101	96-100

CALIBRATION

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

The NIBSC/WHO 1st International Standard for human GM-CSF 88/646 (Human, rDNA-derived) was evaluated in this assay.

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

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

SAMPLE VALUES

Serum/Plasma - Forty serum and plasma samples from apparently healthy volunteers were evaluated for the presence of human GM-CSF in this assay. All samples measured less than the lowest Human GM-CSF Standard, 7.8 pg/mL. No medical histories were available for the donors used in this study.

Cell Culture Supernates - Human peripheral blood mononuclear cells (1 x 10^6 cells/mL) were cultured in RPMI supplemented with 10% fetal bovine serum, 50 µM β-mercaptoethanol, 2 mM L-glutamine, 100 U/mL penicillin, and 100 µg/mL streptomycin sulfate and cultured unstimulated or stimulated with 10 µg/mL PHA. Aliquots of the cell culture supernates were removed on days 1 and 5 and assayed for levels of human GM-CSF.

Condition	Day 1 (pg/mL)	Day 5 (pg/mL)
Unstimulated	39	43
Stimulated	71	31

SPECIFICITY

This assay recognizes natural and recombinant human GM-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 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
IL-3
IL-3 Rα
IL-4
IL-4 Rα
IL-5
IL-5 Rα
M-CSF

Recombinant mouse:

GM-CSF
IL-3
IL-4
IL-5

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