Quantikine® ELISA

Mouse GM-CSF Immunoassay

Catalog Number MGM00
SMGM00
PMGM00

For the quantitative determination of mouse Granulocyte Macrophage Colony Stimulating Factor (GM-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.

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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 mouse GM-CSF shares 54% and 69% amino acid sequence identity with human and rat GM-CSF, respectively (8). Rat GM-CSF is known to be active on mouse cells, while mouse GM-CSF has limited activity on rat cells (9).

The high-affinity receptor for GM-CSF is composed of a 50 kDa ligand binding alpha subunit (GM-CSF Rα) (10) and a 120 kDa signal transducing Common beta Chain (βc) (11, 12). 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 (13, 14). 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 (14). GM-CSF may additionally utilize Syndecan-2 as a co-receptor (15).

A number of functions have been attributed to GM-CSF. It induces monocyte, neutrophil, and eosinophil production from $CD34^+$ stem cell precursors (16, 17). It can act in concert with IL-4 or Flt-3 Ligand to induce the development and maintenance of myeloid and dermal dendritic cells (17-21). It also acts as a neutrophil and dendritic cell chemoattractant (6, 22). 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, 23-27). In addition, it cooperates with G-CSF in promoting tumor cell proliferation and invasion (28).

The Quantikine® Mouse GM-CSF Immunoassay is a 4.5 hour solid phase ELISA designed to measure mouse GM-CSF in cell culture supernates and serum. It contains *E. coli*-expressed recombinant mouse GM-CSF and antibodies raised against the recombinant factor. This immunoassay has been shown to quantitate the recombinant mouse GM-CSF accurately. Results obtained using natural mouse GM-CSF showed dose-response 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 natural mouse GM-CSF.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. A polyclonal antibody specific for mouse GM-CSF has been pre-coated onto a microplate. Standards, control, 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 polyclonal antibody specific for mouse 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. 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 GM-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, 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.
- For best results, pipette reagents and samples into the center of each well.
- It is recommended that the samples be pipetted within 15 minutes.
- 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 #	CATALOG # MGM00	CATALOG # SMGM00	DESCRIPTION	STORAGE OF OPENED/ RECONSTITUTED MATERIAL
Mouse GM-CSF Microplates	890412	2 plates	6 plates	96 well polystyrene microplates (12 strips of 8 wells) coated with a polyclonal antibody specific for mouse 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.*
Mouse GM-CSF Standard	890405	1 vial	3 vials	Recombinant mouse 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.*
Mouse GM-CSF Control	890226	1 vial	3 vials	Recombinant mouse GM-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 GM-CSF Conjugate	890005	1 vial	3 vials	23 mL/vial of a polyclonal antibody specific for mouse GM-CSF conjugated to horseradish peroxidase with preservatives.	May be stored for up to 1 month at 2-8 °C.*
Assay Diluent RD1W	895038	1 vial	3 vials	12 mL/vial of a buffered protein base with preservatives.	
Calibrator Diluent RD5T	895175	1 vial	3 vials	21 mL/vial of a buffered protein base with preservatives.	
Wash Buffer Concentrate	895003	2 vials	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	3 vials	12 mL/vial of stabilized hydrogen peroxide.	
Color Reagent B	895001	1 vial	3 vials	12 mL/vial of stabilized chromogen (tetramethylbenzidine).	
Stop Solution	895174	1 vial	3 vials	23 mL/vial of diluted hydrochloric acid.	
Plate Sealers	N/A	8 strips	24 strips	Adhesive strips.	

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

MGM00 contains sufficient materials to run ELISAs on two 96 well plates.
SMGM00 (SixPak) contains sufficient materials to run ELISAs on six 96 well plates.

This kit is also available in a PharmPak (R&D Systems®, Catalog # PMGM00). 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.

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. 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 - 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.*

REAGENT PREPARATION

Bring all reagents to room temperature before use.

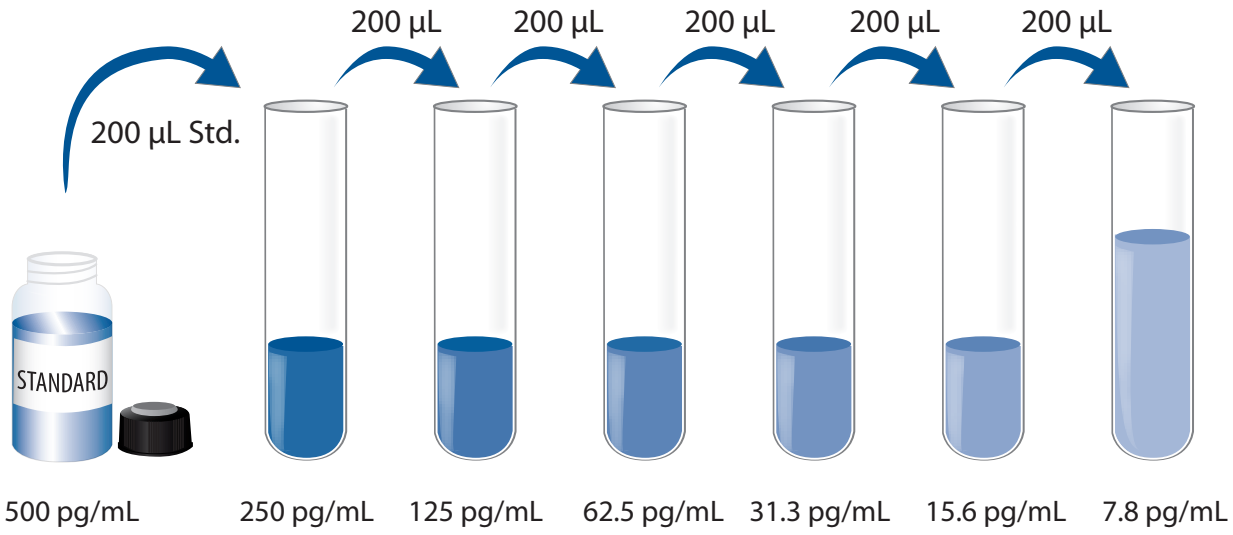
Mouse GM-CSF Control - Reconstitute the control with 1.0 mL deionized or distilled water. Mix thoroughly. Assay the control undiluted.

Wash Buffer - If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. To prepare enough Wash Buffer for one plate, 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 GM-CSF Standard - **Refer to the vial label for reconstitution volume.** Reconstitute the Mouse GM-CSF Standard with Calibrator Diluent RD5T. Do not substitute other diluents. This reconstitution produces a stock solution of 500 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 RD5T into each tube. Use the stock solution to produce a dilution series (below). Mix each tube thoroughly before the next transfer. The undiluted Mouse GM-CSF Standard (500 pg/mL) serves as the high standard. Calibrator Diluent RD5T 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 control be assayed in duplicate.

1. Prepare reagents, standards, control, and samples 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 RD1W 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 GM-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.

CALCULATION OF RESULTS

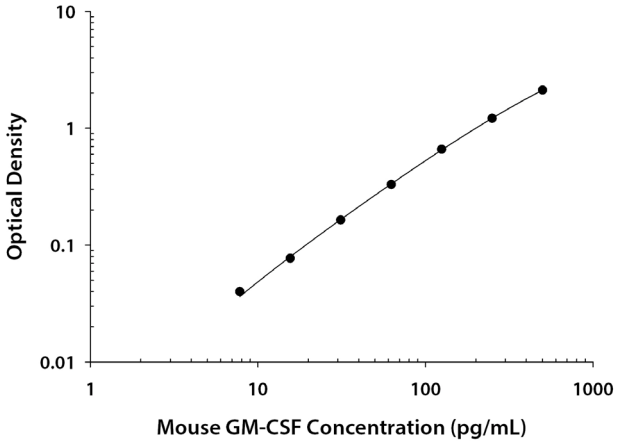
Average the duplicate readings for each standard, control, and sample and subtract the average zero standard optical density (O.D.).

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 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

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	0.035 0.039	0.037	—
7.8	0.074 0.080	0.077	0.040
15.6	0.113 0.116	0.114	0.077
31.3	0.197 0.205	0.201	0.164
62.5	0.359 0.375	0.367	0.330
125	0.685 0.707	0.696	0.659
250	1.221 1.279	1.250	1.213
500	2.137 2.170	2.154	2.117

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)	18.5	67.2	219	20.0	71.7	243
Standard deviation	0.7	2.1	6.9	1.0	3.8	11.2
CV (%)	3.8	3.1	3.2	5.0	5.3	4.6

RECOVERY

The recovery of mouse GM-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=8)	96	82-114%
Serum (n=7)	94	82-112%

LINEARITY

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

Samples	Dilution	Observed (pg/mL)	Expected (pg/mL)	$\frac{\text{Observed}}{\text{Expected}} \times 100$
Cell culture supernates	Neat	338	———	———
	1:2	175	169	104
	1:4	85	84	101
	1:8	43	42	102
	1:16	21	21	100
Serum	Spiked	406	———	———
	1:2	209	203	103
	1:4	109	102	107
	1:8	54	51	106
	1:16	28	25	112

SENSITIVITY

Twenty-five assays were evaluated and the minimum detectable dose (MDD) of mouse GM-CSF ranged from 0.4-5.8 pg/mL. The mean MDD was 1.8 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 GM-CSF produced at R&D Systems®.

The NIBSC/WHO interim reference mouse GM-CSF preparation 91/658, which was intended as a bioassay standard, was evaluated in this kit. Each ampule contains a nominal 1.0 µg of recombinant mouse GM-CSF and was assigned an arbitrary unitage of 100,000 U/ampule.

NIBSC/WHO 91/658: 1 Unit of standard = 6.6 pg of Quantikine® Mouse GM-CSF

SAMPLE VALUES

Serum - Forty samples were evaluated for detectable levels of mouse GM-CSF in the assay. All samples measured less than the lowest Mouse GM-CSF Standard, 7.8 pg/mL.

Cell Culture Supernates:
Mouse splenocytes (2 x 10^6 cells/mL) were stimulated with LPS for 3 days. The cell culture supernate was removed, assayed for mouse GM-CSF, and measured 92 pg/mL.

Mouse heart or lung conditioned media (1 heart or 1 lung; 1-2 mm pieces in 10 mL of medium) was collected after culturing for 5 days. The cell culture supernates were removed, assayed for mouse GM-CSF, and measured 628 pg/mL (heart) and 17 ng/mL (lung).

SPECIFICITY

This assay recognizes natural and recombinant mouse 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 prepared at 50 ng/mL in a mid-range mouse GM-CSF control were assayed for interference.

Recombinant mouse:

C10
G-CSF
IL-1α
IL-1β
IL-2
IL-3
IL-4
IL-5
IL-6
IL-7
IL-9
IL-10
IL-13
JE
LIF
M-CSF
MIP-1α
MIP-1β
MIP-2
SCF
TNF-α

Recombinant human:

G-CSF
GM-CSF
M-CSF

Recombinant rat GM-CSF cross-reacts approximately 0.45% in this assay.

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