Quantikine® ELISA

Mouse CCL3/MIP-1α Immunoassay

Catalog Number MMA00
SMMA00
PMMA00

For the quantitative determination of mouse Macrophage Inflammatory Protein 1 alpha (MIP-1α) concentrations in cell culture supernates and serum.

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.
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INTRODUCTION

Macrophage Inflammatory Protein 1α and 1β (MIP-1α and 1b), two closely related but distinct proteins, were originally co-purified from medium conditioned by an LPS-stimulated mouse macrophage cell line (1). The MIP-1 proteins are members of the β or CC subfamily of chemokines, a large superfamily of small, inducible, secreted, pro-inflammatory cytokines that are involved in a variety of immune and inflammatory responses. Additional chemokine subfamilies, defined by the arrangement of the conserved cysteine (C) residues of the mature proteins, include the α or CXC and the γ or C subfamilies. Chemokines share from 20 to greater than 90 percent amino acid sequence identity. Most chemokines have been shown to act primarily as chemoattractants and activators of specific types of leukocytes (2-4).

Mouse MIP-1α cDNA encodes a 92 amino acid (aa) residue precursor protein with a 23 aa residue signal peptide that is cleaved to generate the secreted mature protein (5). Mature mouse MIP-1α shares approximately 70% amino acid identity with mouse MIP-1β. MIP-1α expression can be induced in a variety of cell types, including T cells, B cells, monocytes, mast cells, neutrophils, Langerhans cells, astrocytes, endothelial cells, fibroblasts and smooth muscle cells (3-9). The gene for MIP-1α has been mapped to mouse chromosome 11, along with all other mouse b chemokine genes (3).

The biological activities of MIP-1α overlap with those of other β chemokines. MIP-1α is a monocyte chemoattractant (2, 3). It has also been reported to have differential chemoattractant and pro-adhesive effects on T-lymphocytes (10, 11), NK cells (12), cytotoxic T cells, B cells (13), basophils (14), and eosinophils (15). MIP-1α and other β chemokines have been shown to enhance some lymphocyte effector functions (16) and to induce the generation of killer cells from $CD56^+$ cells (17). MIP-1α, MIP-1β, and RANTES have been implicated as the major human HIV-suppressive factors produced by human $CD8^+$ T cells (18). MIP-1α has been identified as a stem cell inhibitor (SCI) that can inhibit the proliferation of hematopoietic progenitor cells both *in vitro* and *in vivo* (19-22).

The Quantikine Mouse MIP-1α Immunoassay is a 4.5 hour solid phase ELISA designed to measure mouse MIP-1α levels in cell culture supernates and serum. It contains *E. coli*-expressed recombinant mouse MIP-1α and antibodies raised against the recombinant protein. Results obtained for naturally occurring mouse MIP-1α 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 of natural mouse MIP-1α.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. A polyclonal antibody specific for mouse MIP-1α has been pre-coated onto a microplate. Standards, Control, and samples are pipetted into the wells and any mouse MIP-1α present is bound by the immobilized antibody. After washing away any unbound substances, an enzyme-linked polyclonal antibody specific for mouse MIP-1α 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 mouse MIP-1α 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 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 # MMA00	CATALOG # SMMA00	DESCRIPTION	STORAGE OF OPENED/ RECONSTITUTED MATERIAL
Mouse MIP-1α Microplates	890348	2 plates	6 plates	96 well microplates (12 strips of 8 wells) coated with a polyclonal antibody specific for mouse MIP-1α.	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 MIP-1α Standard	890350	1 vial	3 vials	Recombinant mouse MIP-1α 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.
Mouse MIP-1α Control	890351	1 vial	3 vials	Recombinant mouse MIP-1α in a buffered protein base with preservatives; lyophilized. The concentration range of mouse MIP-1α after reconstitution is shown on the vial label. The assay value of the Control should be within the range specified on the label.	
Mouse MIP-1α Conjugate	890349	1 vial	3 vials	23 mL/vial of a polyclonal antibody against mouse MIP-1α conjugated to horseradish peroxidase with preservatives.	May be stored for up to 1 month at 2-8 °C.*
Assay Diluent RD1-21	895215	1 vial	3 vials	12 mL/vial of a buffered protein solution with preservatives.	
Calibrator Diluent RD5Z	895206	1 vial	3 vials	21 mL/vial of a buffered protein solution 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	4 adhesive strips.	

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

MMA00 contains sufficient materials to run ELISAs on two 96 well plates.
SMMA00 (SixPak) contains sufficient materials to run ELISAs on six 96 well plates.

This kit is also available in a PharmPak (R&D Systems, Catalog # PMMA00). 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.

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 are not suitable for use in this assay.*

REAGENT PREPARATION

Bring all reagents to room temperature before use.

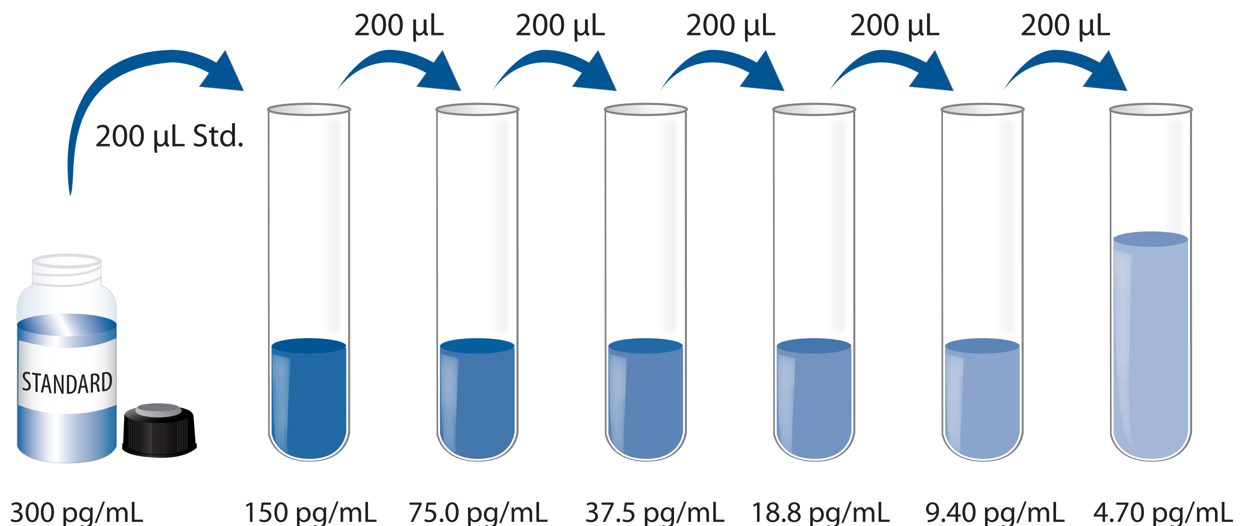
Mouse MIP-1α Control - Reconstitute the Control with 1.0 mL of deionized or distilled water. Assay the Control undiluted.

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 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 MIP-1α Standard - **Refer to the vial label for reconstitution volume.** Reconstitute the mouse MIP-1α Standard with Calibrator Diluent RD5Z. This reconstitution produces a stock solution of 300 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 RD5Z into each tube. Use the stock solution to produce a 2-fold dilution series (below). Mix each tube thoroughly before the next transfer. The undiluted Mouse MIP-1α Standard serves as the high standard (300 pg/mL). Calibrator Diluent RD5Z 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 and standard dilutions as directed by 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 RD1-21 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. A plate layout is provided to record standards and samples assayed.
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 MIP-1α 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.

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CALCULATION OF RESULTS

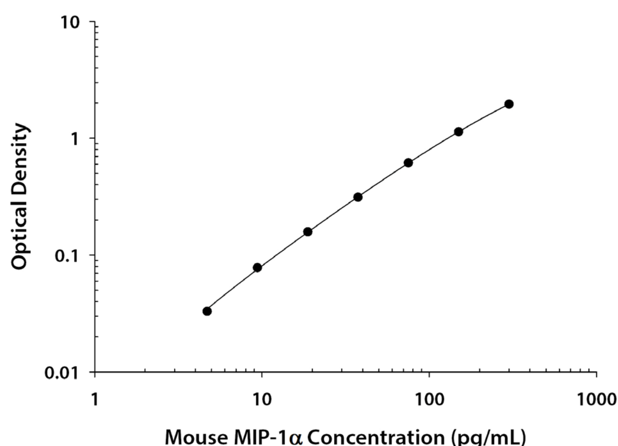
Average the duplicate readings for each standard, control, and sample and subtract the average zero standard optical density (O.D.).

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 MIP-1α 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 prior to the assay, their measured concentrations 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	0.059 0.052	0.056	—
4.70	0.091 0.087	0.089	0.033
9.40	0.138 0.130	0.134	0.078
18.8	0.209 0.219	0.214	0.158
37.5	0.365 0.371	0.368	0.312
75.0	0.678 0.665	0.672	0.616
150	1.234 1.150	1.192	1.136
300	2.063 1.956	2.010	1.954

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.

	Intra-Assay Precision			Inter-Assay Precision		
Sample	**1**	**2**	**3**	**1**	**2**	**3**
n	20	20	20	20	20	20
Mean (pg/mL)	13.7	86.0	184	13.8	83.0	179
Standard deviation	0.6	3.1	5.0	0.9	5.2	12
CV (%)	4.4	3.6	2.7	6.5	6.3	6.7

RECOVERY

The recovery of mouse MIP-1α spiked to three levels throughout the range of the assay in various matrices was evaluated.

Sample Type	Average % Recovery	Range
Cell culture supernates (n=5)	105	95-114%
Serum (n=9)	97	85-108%

LINEARITY

To assess the linearity of the assay, samples containing and/or spiked with various concentrations of mouse MIP-1α in each matrix were diluted with Calibrator Diluent and then assayed.

		Cell culture supernates (n=8)	Serum (n=8)
1:2	Average % of Expected	100	103
	Range (%)	97-107	99-106
1:4	Average % of Expected	96	104
	Range (%)	92-101	98-110
1:8	Average % of Expected	93	105
	Range (%)	90-98	98-111
1:16	Average % of Expected	87	103
	Range (%)	81-93	93-112

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SENSITIVITY

The minimum detectable dose (MDD) of mouse MIP-1α is typically less than 1.5 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 *E. coli*-expressed recombinant mouse MIP-1α produced at R&D Systems.

SAMPLE VALUES

Serum - Forty samples were evaluated for detectable levels of mouse MIP-1α in this assay. Thirty-seven samples read below the lowest standard, 4.7 pg/mL. Three samples read 5.3 pg/mL, 6.2 pg/mL, and 8.9 pg/mL, respectively.

Cell Culture Supernates:
J774A.1 mouse reticulum cell sarcoma macrophage cells (1 x 10^6 cells/mL) were cultured for 2 days in RPMI containing 10% fetal calf serum, 500 ng/mL recombinant mouse IFN-γ and stimulated with 1 µg/mL LPS. The cell culture supernate was removed, assayed for mouse MIP-1α, and measured 65 ng/mL.

Mouse lung conditioned media (1 lung, 1-2 mm pieces in 10 mL of medium) was cultured for 5 days in RPMI supplemented with 10% fetal calf serum. The cell culture supernate was removed, assayed for mouse MIP-1α, and measured 31 ng/mL.

SPECIFICITY

This assay recognizes natural and recombinant mouse MIP-1α.

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 MIP-1α control were assayed for interference. No significant cross-reactivity or interference was observed.

Recombinant mouse:

C10
G-CSF
GM-CSF
IFN-γ
IL-1α
IL-1β
IL-2
IL-3
IL-4
IL-5
IL-6
IL-7
IL-9
IL-10
IL-10 sR
IL-12
IL-13
JE/MCP-1
KC
Leptin
LIF
MARC
M-CSF
MIP-2
SCF
TNF-α
Tpo
VEGF

Recombinant human:

GROα
GROβ
IL-8
MIP-1α
RANTES

Some cross-reactivity was observed with the following:

Recombinant Factor	Concentration Tested (pg/mL)	Observed Value (pg/mL)	% Cross-Reactivity
Mouse MIP-1β	50,000	20.0	0.04
Human MIP-1β	50,000	142	0.28

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PLATE LAYOUT

Use this plate layout to record standards and samples assayed.

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NOTES

NOTES

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