Quantikine® ELISA

Mouse CXCL2/MIP-2 Immunoassay

Catalog Number MM200
SMM200
PMM200

For the quantitative determination of mouse Macrophage Inflammatory Protein 2 (MIP-2) 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

Mouse Macrophage Inflammatory Protein-2 (MIP-2), also known as CXCL2, was originally identified as a heparin-binding protein secreted by an LPS-stimulated mouse macrophage cell line (1). A cDNA clone encoding the protein was isolated from this cell line and characterized (2). Based on its protein and DNA sequences, mouse MIP-2 was classified as a member of the alpha (CXC) chemokine family of inflammatory and immunoregulatory cytokines (3).

Mouse MIP-2 cDNA encodes a 100 amino acid residue precursor protein from which the amino-terminal 27 amino acid residues are cleaved to generate the mature mouse MIP-2. The protein sequence of mouse MIP-2 shows approximately 63% identity to that of mouse KC, another mouse alpha chemokine. Mouse MIP-2 is also 60% identical to human GROβ and GROγ (2). Based on these protein sequence similarities, it is likely that mouse KC and MIP-2 are homologs of human GROα, β and γ chemokines. Since chemokines with protein sequence homology to human IL-8 have not been identified in mice, it has been suggested that the mouse KC and MIP-2 are functional homologs of human IL-8 in mice (3, 4). A putative mouse homolog of the human IL-8 receptor beta (IL-8 Rβ) has also been cloned. This receptor shows 71% identity to human IL-8 Rβ and 68% identity to human IL-8 Rα. Both mouse KC and MIP-2 bind mouse IL-8 Rβ with high affinity (5).

Like human IL-8, mouse MIP-2 exhibits potent neutrophil chemotactic activity and may be a key mediator of neutrophil recruitment in response to tissue injury and infection (3, 4). Increased MIP-2 expression has been found to be associated with neutrophil influx in various inflammatory conditions (6-10).

The Quantikine® Mouse CXCL2/MIP-2 Immunoassay is a 4.5 hour solid phase ELISA designed to measure mouse MIP-2 levels in cell culture supernates and serum. It contains *E. coli*-expressed recombinant mouse MIP-2 and antibodies raised against the recombinant factor. This immunoassay has been shown to quantitate the recombinant mouse MIP-2 accurately. Results obtained using natural mouse MIP-2 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 MIP-2.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. A polyclonal antibody specific for mouse MIP-2 has been pre-coated onto a microplate. Standards, control, and samples are pipetted into the wells and any MIP-2 present is bound by the immobilized antibody. After washing away any unbound substances, an enzyme-linked polyclonal antibody specific for mouse MIP-2 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 MIP-2 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.
- 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.

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 SDS 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 #	CATALOG # MM200	CATALOG # SMM200	DESCRIPTION	STORAGE OF OPENED/ RECONSTITUTED MATERIAL
Mouse MIP-2 Microplate	890469	2 plates	6 plates	96 well polystyrene microplates (12 strips of 8 wells) coated with a polyclonal antibody specific for mouse MIP-2.	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-2 Standard	890471	1 vial	3 vials	Recombinant mouse MIP-2 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 MIP-2 Control	890464	1 vial	3 vials	Recombinant mouse MIP-2 in a buffered protein base with preservatives; lyophilized. The assay value of the control should be within the range specified on the label.	
Mouse MIP-2 Conjugate	890470	1 vial	3 vials	23 mL/vial of a polyclonal antibody specific for mouse MIP-2 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 solution with preservatives.	
Calibrator Diluent RD5Z	895206	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.

MM200 contains sufficient materials to run ELISAs on two 96 well plates.
SMM200 (SixPak) contains sufficient materials to run ELISAs on six 96 well plates.

This kit is also available in a PharmPak (R&D Systems®, Catalog # PMM200). PharmPaks contain sufficient materials to run ELISAs on 50 microplates. Specific vial counts of each component may vary. Refer to the PharmPak Contents section for specific vial counts.

PHARMPAK CONTENTS

Each PharmPak contains reagents sufficient for the assay of 50 microplates (96 wells/plate). The package inserts supplied are the same as those supplied in the single kit packs and because of this, a few minor differences related to the number of reagents and their container sizes should be noted.

- Sufficient material is supplied to perform at least 50 standard curves; reuse of each vial may be required. The number of vials, and the number of standard curves obtained per vial will vary with the analyte.
- Wash Buffer 25X Concentrate is bulk packed in 125 mL bottles containing 100 mL, and not in the glass vials described in the package insert. **Note:** *Additional wash buffer is available for purchase (R&D Systems®, Catalog # WA126).*

The reagents provided in this PharmPak are detailed below.

PART	PART #	QUANTITY
Mouse MIP-2 Microplate	890469	50 plates
Mouse MIP-2 Standard	890471	25 vials
Mouse MIP-2 Control	890464	25 vials
Mouse MIP-2 Conjugate	890470	25 vials
Assay Diluent RD1W	895038	25 vials
Calibrator Diluent RD5Z	895206	25 vials
Wash Buffer Concentrate, 25X	895126	9 bottles
Color Reagent A	895000	25 vials
Color Reagent B	895001	25 vials
Stop Solution	895174	25 vials
Plate Sealers	N/A	100 sheets
Package Inserts	752304	2 booklets

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.

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.

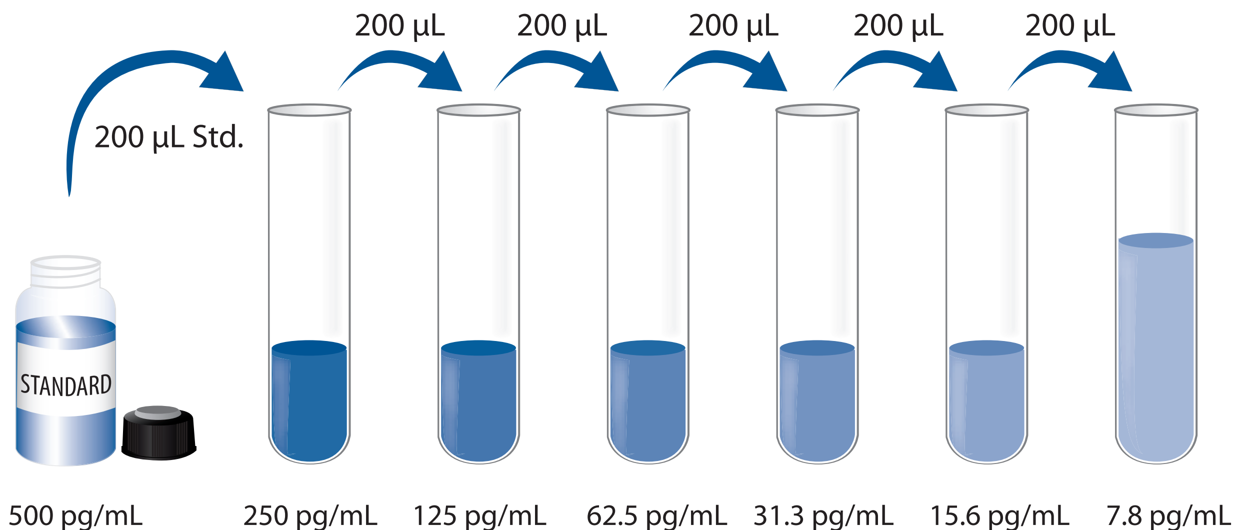
Mouse MIP-2 Control - Reconstitute the control with 1.0 mL deionized or distilled water. Mix thoroughly. Assay the control undiluted.

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 Wash Buffer Concentrate to 480 mL of 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-2 Standard - **Refer to the vial label for reconstitution volume.** Reconstitute the Mouse MIP-2 Standard with Calibrator Diluent RD5Z. 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 RD5Z into each tube. Use the stock solution to produce a dilution series (below). Mix each tube thoroughly before the next transfer. The undiluted Mouse MIP-2 Standard (500 pg/mL) serves as the high standard. Calibrator Diluent RD5Z serves as the zero standard (0 pg/mL).



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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 all reagents, working standards, control, and samples 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 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. 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-2 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

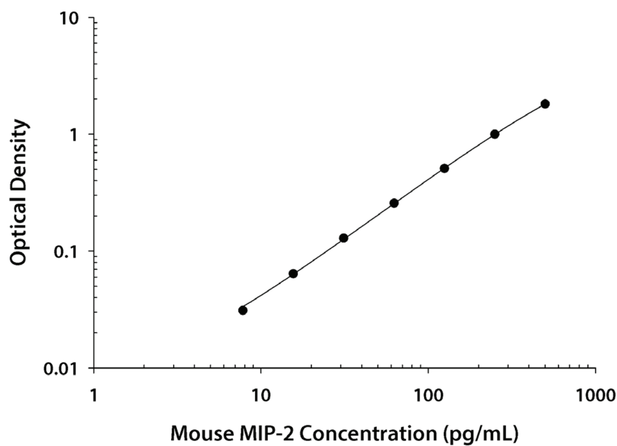
Average the duplicate readings for each standard, control, and sample and subtract the average zero standard optical density (O.D.).

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 MIP-2 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	0.012 0.013	0.012	—
7.8	0.043 0.043	0.043	0.031
15.6	0.078 0.074	0.076	0.064
31.3	0.139 0.143	0.141	0.129
62.5	0.271 0.267	0.269	0.257
125	0.521 0.518	0.520	0.508
250	1.016 1.003	1.010	0.998
500	1.822 1.824	1.823	1.811

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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)	17.7	53.9	363	16.9	52.1	346
Standard deviation	0.5	1.3	7.3	1.0	2.3	15.4
CV (%)	2.8	2.4	2.0	5.9	4.4	4.5

RECOVERY

The recovery of mouse MIP-2 spiked to three levels throughout the range of the assay in various matrices was evaluated.

Sample Type	Average % Recovery	Range
Cell culture supernates (n=10)	96	84-110%
Serum (n=14)	100	94-116%

LINEARITY

To assess the linearity of the assay, five or more samples containing and/or spiked with various concentrations of mouse MIP-2 in each matrix were diluted with calibrator diluent and then assayed.

Sample	Dilution	Observed (pg/mL)	Expected (pg/mL)	Observed/Expected x 100
Cell culture supernates	Neat	317	—	—
	1:2	155	158	98
	1:4	79	79	100
	1:8	39	40	98
	1:16	19	20	95
Serum	Spiked	350	—	—
	1:2	177	175	101
	1:4	92	88	105
	1:8	46	44	105
	1:16	22	22	100

SENSITIVITY

The minimum detectable dose (MDD) of mouse MIP-2 is typically less than 1.5 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 MIP-2 produced at R&D Systems®.

SAMPLE VALUES

Serum - Serum samples were evaluated for detectable levels of mouse MIP-2 in this assay.

Sample Type	Mean of Detectable (pg/mL)	% Detectable	Range (pg/mL)
Serum (n=40)	19.34	78	ND-50.72

ND=Non-detectable

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 bovine serum, 500 ng/mL recombinant mouse IFN-γ and stimulated with 1.0 µg/mL LPS. An aliquot of the cell culture supernate was removed, assayed for mouse MIP-2, and measured 55 ng/mL.

Mouse lung, cut into 1-2 mm pieces, was cultured for 5 days in 10 mL of medium. An aliquot of the cell culture supernate was removed, assayed for mouse MIP-2, and measured 135 ng/mL.

SPECIFICITY

This assay recognizes natural and recombinant mouse MIP-2.

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-2 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 R
IL-12
IL-13
JE/MCP-1
LIF
M-CSF
MIP-1α
MIP-1β
SCF
TNF-α
Tpo
VEGF

Recombinant human:

GROα
GROβ
IL-8
MIP-1α
MIP-1β

Some cross-reactivity was observed with the following:

Recombinant Factor	Concentration Tested (pg/mL)	Observed Value (pg/mL)	% Cross-reactivity
mouse KC	50,000	15.9	0.03
human RANTES	50,000	26.8	0.05

REFERENCES

1. Wolpe, S.D. *et al.* (1989) Proc. Natl. Acad. Sci. USA **86**:612.
2. Tekamp-Olson, P. *et al.* (1990) J. Exp. Med. **172**:911.
3. Schall, T. (1994) in *The Cytokine Handbook*, 2nd ed., A. Thomson, ed., Academic Press, New York, p. 419.
4. Driscoll, K.E. (1994) Exp. Lung Res. **20**:473.
5. Heinrich, J. and R. Bravo (1995) J. Biol. Chem. **270**:4987.
6. Standiford, T.J. *et al.* (1996) J. Leukoc. Biol. **59**:24.
7. Su, Y.H. *et al.* (1996) J. Virol. **70**:1277.
8. Greenberger, M.J. *et al.* (1996) J. Infect. Dis. **173**:159.
9. Seebach, J. *et al.* (1995) J. Immunol. **155**:4367.
10. Standiford, T.J. *et al.* (1995) J. Immunol. **155**:2222.

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PLATE LAYOUT

Use this plate layout to record standards and samples assayed.

	A	B	C	D	E	F	G	H
12								
11								
10								
9								
8								
7								
6								
5								
4								
3								
2								
1								

NOTES

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