Quantikine® ELISA

Mouse CCL20/MIP-3α Immunoassay

Catalog Number MCC200

For the quantitative determination of mouse Macrophage Inflammatory Protein 3 alpha (MIP-3α) concentrations in cell culture supernates, serum, and plasma.

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 MIP-3α, otherwise known as CCL20, LARC, and Exodus, is an 8 kDa protein belonging to the β-, or CC chemokine sub-family (1- 4). It is synthesized as a 97 amino acid (aa) precursor that contains a 27 aa signal sequence plus a 70 aa mature segment (5). Mature mouse MIP-3α contains no potential N-linked glycosylation sites. Although human MIP-3α is suggested to contain an O-linked ribose, rodent MIP-3α does not contain any proline rich motifs that might indicate glycosylation (6). MIP-3α is known to exist in multiple isoforms (7, 8). An alternate splice form lacking the N-terminal alanine exists in both mouse and human. In addition, human MIP-3α apparently undergoes proteolytic processing at the C-terminus to generate peptides that are shorter by one, three, and six amino acid (aa) residues (6). All isoforms are equally bioactive. It is possible that mouse MIP-3α may be processed similarly. The mature region of mouse MIP-3α shares 73% and 70% aa sequence identity with mature rat and human MIP-3α, respectively (8-12). Among mouse β-chemokines, MIP-3α is distantly related to MIP-3β sharing 34% aa sequence identity (5, 13). Cells known to express MIP-3α include monocytes (11, 12), macrophages, microglia and astrocytes (9), mast cells (14), neutrophils (15), keratinocytes (16, 17), intestinal epithelium (18, 19), osteoblasts (20), fibroblasts (21, 22), endothelial cells (22, 23), and bronchial epithelial cells (24).

The only known receptor for MIP-3α is CCR6 (2, 3, 5), which is expressed on a variety of cells, including neutrophils (25), naive and memory B cells (26), Langerhans and immature dendritic cells (16, 19, 22, 27), and $\alpha_4\beta_7^+$ intestinal plus CLA^+ dermal memory T cells (28). Almost all of these cells are required to migrate to sites of infection or inflammation.

The MIP-3α/CCR6 ligand/receptor pair would appear to provide direction to "first-responders" migrating to sites of bacterial infection (7). MIP-3α is an inducible chemokine that is the product of LPS and/or TNF-α/IL-1β stimulation (3, 7, 22). Bacterial wall LPS, in conjunction with bacterially-induced TNF-α, induces the synthesis of MIP-3α in both endothelium and epithelium. From endothelium, the chemokine promotes lymphocyte adhesion to vessel walls (23). In tissue, it directs inflammatory cell migration to sites of epithelial insult, either in the intestine or skin (16, 19, 28). From here, immature dendritic cells will engage the antigen, down-modulate CCR6, upregulate CCR7 and migrate to regional lymph nodes. Alternatively, antigen-responsive memory lymphocytes will be chemoattracted to sites of antigen concentration, initiating antibody or T cell responses.

The Quantikine® Mouse CCL20/MIP-3α Immunoassay is a 4.5 hour solid-phase ELISA designed to measure mouse MIP-3α in cell culture supernates, serum, and plasma. It contains *E. coli*-expressed recombinant mouse MIP-3α and antibodies raised against the recombinant factor. This immunoassay has been shown to accurately quantitate the recombinant factor. Results obtained using natural mouse MIP-3α 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 natural mouse MIP-3α.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for mouse MIP-3α has been pre-coated onto a microplate. Standards, control, and samples are pipetted into the wells and any MIP-3α present is bound by the immobilized antibody. After washing away any unbound substances, an enzyme-linked monoclonal antibody specific for mouse MIP-3α 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-3α 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.

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
Mouse MIP-3α Microplate	892544	96 well polystyrene microplate (12 strips of 8 wells) coated with a monoclonal antibody specific for mouse MIP-3α.	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-3α Standard	892546	2 vials of recombinant mouse MIP-3α in a buffered protein base with preservatives; lyophilized. *Refer to the vial label for reconstitution volume.*	Use a new standard and control for each assay. Discard after use.
Mouse MIP-3α Control	892547	2 vials of recombinant mouse MIP-3α 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-3α Conjugate	892545	12 mL of a monoclonal antibody specific for mouse MIP-3α conjugated to horseradish peroxidase with preservatives.	May be stored for up to 1 month at 2-8 °C.*
Assay Diluent RD1W	895038	12 mL of a buffered protein base with preservatives.	
Calibrator Diluent RD5T	895175	21 mL of a buffered protein base with preservatives.	
Wash Buffer Concentrate	895003	21 mL of a 25-fold concentrated solution of buffered surfactant with preservative. *May turn yellow over time.*	
Color Reagent A	895000	12 mL of stabilized hydrogen peroxide.	
Color Reagent B	895001	12 mL of stabilized chromogen (tetramethylbenzidine).	
Stop Solution	895174	23 mL of diluted hydrochloric acid.	
Plate Sealers	N/A	4 adhesive strips.	

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

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.
- Horizontal orbital microplate shaker (0.12" orbit) capable of maintaining a speed of 500 ± 50 rpm.
- **Polypropylene** 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. Refer to the SDS 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. 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.

Plasma - Collect plasma using heparin as a anticoagulant. Centrifuge for 20 minutes at 2000 x g within 30 minutes of collection. Assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

Note: *EDTA plasma is not recommended for use in this assay.*
Citrate plasma has not been validated for use in this assay.
Grossly hemolyzed or lipemic samples may not suitable for use in this assay.

REAGENT PREPARATION

Bring all reagents to room temperature before use.

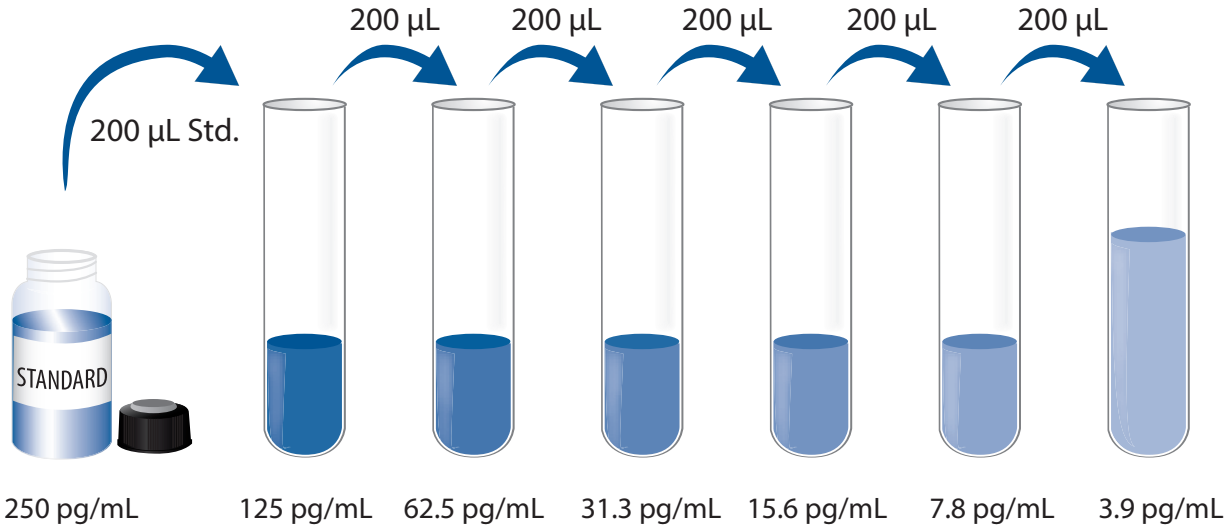
Mouse MIP-3α Control - Reconstitute the control with 1.0 mL of deionized or distilled water. Mix thoroughly. Assay the control undiluted.

Wash Buffer - If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. Add 20 mL of 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-3α Standard - **Refer to the vial label for reconstitution volume.** Reconstitute the Mouse MIP-3α Standard with Calibrator Diluent RD5T. Do not substitute other diluents. This reconstitution produces a stock solution of 250 pg/mL. Allow the standard to sit for a minimum of 5 minutes with gentle mixing prior to making dilutions.

Use polypropylene tubes. 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 MIP-3α Standard (250 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 standards, control, and samples be assayed in duplicate.

1. Prepare all reagents, standard dilutions, 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 the center of each well.
4. Add 50 µL of standard, control, or sample per well. Cover with the adhesive strip provided. Incubate for 2 hours at room temperature on a horizontal orbital microplate shaker (0.12" orbit) set at 500 ± 50 rpm.
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-3α Conjugate to each well. Cover with a new adhesive strip. Incubate for 2 hours at room temperature on the shaker.
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 **on the benchtop. 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

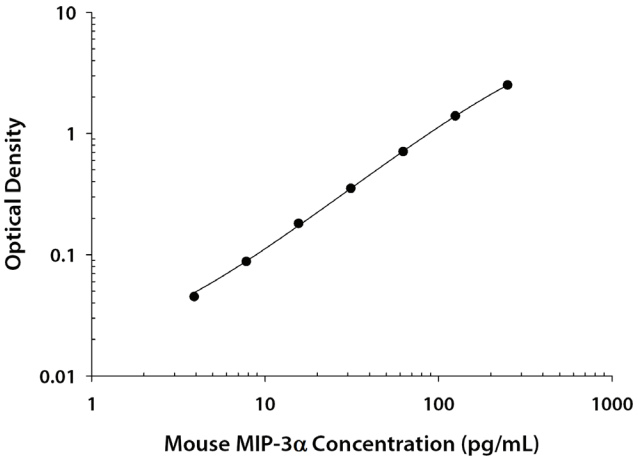
Average the duplicate readings for each standard, control, and sample and subtract the average zero standard optical density (O.D.).

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-3α 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	0.033 0.033	0.033	—
3.9	0.078 0.079	0.078	0.045
7.8	0.120 0.122	0.121	0.088
15.6	0.212 0.216	0.214	0.181
31.3	0.385 0.386	0.386	0.353
62.5	0.738 0.745	0.742	0.709
125	1.403 1.457	1.430	1.397
250	2.510 2.579	2.544	2.511

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

	Intra-Assay Precision			Inter-Assay Precision		
Sample	**1**	**2**	**3**	**1**	**2**	**3**
n	20	20	20	23	23	23
Mean (pg/mL)	10	43	118	11	40	112
Standard deviation	0.6	1.7	5.9	1.0	3.6	7.5
CV (%)	6.0	4.0	5.0	9.1	9.0	6.7

RECOVERY

The recovery of mouse MIP-3α spiked to three levels throughout the range of the assay in various matrices was evaluated.

Sample Type	Average % Recovery	Range
Cell culture supernates (n=4)	100	96-106%
Serum (n=4)	106	95-119%
Heparin plasma (n=4)	98	85-115%

LINEARITY

To assess the linearity of the assay, samples spiked with high concentrations of mouse MIP-3α were serially diluted with calibrator diluent to produce samples with values within the dynamic range of the assay.

		Cell culture supernates (n=4)	Serum (n=4)	Heparin plasma (n=4)
1:2	Average % of Expected	102	103	108
	Range (%)	97-106	101-106	106-113
1:4	Average % of Expected	102	101	106
	Range (%)	95-106	97-105	103-111
1:8	Average % of Expected	104	103	110
	Range (%)	96-110	99-108	108-111
1:16	Average % of Expected	105	110	112
	Range (%)	98-111	102-114	109-114

SENSITIVITY

Twelve assays were evaluated and the minimum detectable dose (MDD) of mouse MIP-3α ranged from 0.15-0.49 pg/mL. The mean MDD was 0.30 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-3α produced at R&D Systems®.

SAMPLE VALUES

Serum/Plasma - Samples were evaluated for the presence of mouse MIP-3α in this assay.

Sample Type	Mean of Detectable (pg/mL)	% Detectable	Range (pg/mL)
Serum (n=20)	8.1	70	ND-14.6
Heparin plasma (n=20)	8.8	45	ND-22.0

ND=Non-detectable

Cell Culture Supernates - J774A.1 mouse reticulum cell sarcoma macrophage cells (1 x 10^6 cells/mL) were cultured for 4 days in DMEM supplemented with 10% fetal bovine serum and stimulated with 2.5 ng/mL LPS. An aliquot of the cell culture supernate was removed, assayed for mouse MIP-3α, and measured 29 pg/mL.

SPECIFICITY

This assay recognizes natural and recombinant mouse MIP-3α.

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 mouse MIP-3α control were assayed for interference. No significant cross-reactivity or interference was observed.

Recombinant mouse:

6Ckine
C10
CCL28
CTACK
Eotaxin
Eotaxin-2
JE/MCP-1
MCP-5
MDC
MIP-1α
MIP-1β
MIP-1γ
MIP-2
MIP-3β
RANTES
TARC
TECK

Recombinant human:

MIP-3α
MIP-3β

Other recombinants:

cotton rat MIP-2
rat MIP-3α

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