Quantikine® ELISA

Mouse CCL12/MCP-5 Immunoassay

Catalog Number MCC120

For the quantitative determination of mouse Monocyte Chemotactic Protein 5 (MCP-5) concentrations in cell culture supernates, serum, and plasma.

Note: The standard reconstitution method has changed. 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

Mouse MCP-5 is a member of the MCP/eotaxin subfamily within the CC chemokine family of inflammatory and immunoregulatory cytokines (1-3). Mouse MCP-5 cDNA encodes a 104 amino acid (aa) precursor protein with a putative 22 aa signal sequence. The 9.3 kDa, 82 aa mature mouse MCP-5 contains five cysteines and no potential N-linked glycosylation sites (4, 5). A sequence polymorphism in the C-terminal region of mouse MCP-5 from SLJ mice has been reported. This polymorphism results in a premature stop codon that deletes the last nine aa residues of the protein. Mouse MCP-5 shares 64% aa identity with human MCP-1, and also shares 51%, 54%, and 54% aa identity with mouse JE, MCP-2, and MCP-3, respectively (4, 6, 7). Although mouse JE has been regarded as the mouse ortholog of human MCP-1, mouse MCP-5 has higher aa sequence identity with MCP-1 and probably also serves as a structural and functional homolog of human MCP-1 (2, 4). MCP-5 has been mapped to the CC chemokine cluster on mouse chromosome 11, and the protein is expressed in macrophages (4, 5), Kupffer cells (8), smooth muscle cells (4), and mast cells (4).

Recombinant mouse MCP-5 is a potent chemoattractant for monocytes. However, the activities of MCP-5 on eosinophils are contradictory. Whereas some studies show that MCP-5 has chemotactic activity for eosinophils, others show only weak eosinophil chemotactic activity at high MCP-5 concentrations (2, 4, 5, 9).

MCP-5 is an agonist for CCR2, also known as the MCP-1 receptor (5, 10). Given that mouse JE is a more potent agonist for CCR2 however, another as yet unidentified receptor for MCP-5 may exist (5).

The Quantikine® Mouse CCL12/MCP-5 Immunoassay is a 4.5 hour solid phase ELISA designed to measure mouse MCP-5 levels in cell culture supernates, serum, and plasma. It contains *E. coli*-expressed recombinant mouse MCP-5 and antibodies raised against the recombinant factor. Results obtained for naturally occurring mouse MCP-5 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 MCP-5.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for mouse MCP-5 has been pre-coated onto a microplate. Standards, control, and samples are pipetted into the wells and any MCP-5 present is bound by the immobilized antibody. After washing away any unbound substances, an enzyme-linked polyclonal antibody specific for mouse MCP-5 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 MCP-5 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, further 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 #	DESCRIPTION	STORAGE OF OPENED/ RECONSTITUTED MATERIAL
Mouse MCP-5 Microplate	891013	96 well polystyrene microplate (12 strips of 8 wells) coated with a monoclonal antibody specific for mouse MCP-5.	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 MCP-5 Standard	891015	Recombinant mouse MCP-5 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 MCP-5 Control	891016	Recombinant mouse MCP-5 in a buffered protein base with preservatives; lyophilized. The assay value of the control should be within the range specified on the label.	
Mouse MCP-5 Conjugate	891014	12 mL of a polyclonal antibody specific for mouse MCP-5 conjugated to horseradish peroxidase with preservatives.	May be stored for up to 1 month at 2-8 °C.*
Assay Diluent RD1-47	895524	12 mL of a buffered protein base with preservatives.	
Calibrator Diluent RD5-3	895436	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.
- **Polypropylene** test tubes for dilution of standards and samples.

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 EDTA as an 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: *Heparin and citrate plasma have not been validated for use in this assay. Grossly hemolyzed or lipemic samples may be not suitable for use in this assay.*

SAMPLE PREPARATION

Use polypropylene tubes.

Serum and plasma samples require a 2-fold dilution. A suggested 2-fold dilution is 75 µL of sample + 75 µL of Calibrator Diluent RD5-3.

REAGENT PREPARATION

Bring all reagents to room temperature before use.

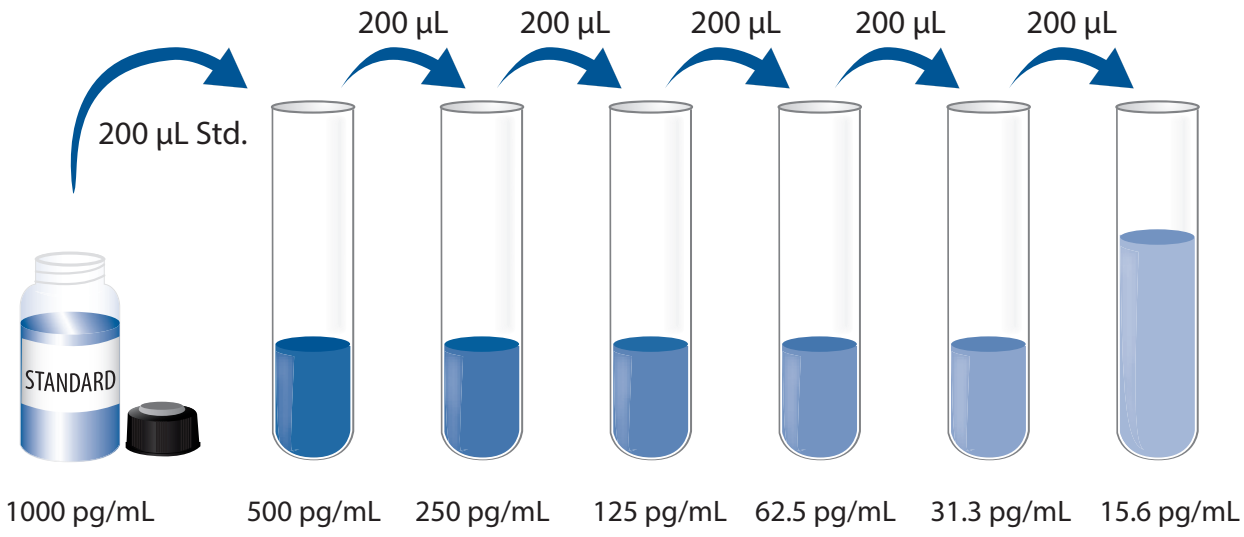
Mouse MCP-5 Control - Reconstitute the control with 1.0 mL of deionized or distilled water. Mix thoroughly. Assay the control undiluted.

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 MCP-5 Standard - **Refer to the vial label for reconstitution volume.** Reconstitute the Mouse MCP-5 Standard with Calibrator Diluent RD5-3. Do not substitute other diluents. This reconstitution produces a stock solution of 1000 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 RD5-3 into each tube. Use the stock solution to produce a dilution series (below). Mix each tube thoroughly before the next transfer. The undiluted Mouse MCP-5 Standard (1000 pg/mL) serves as the high standard. Calibrator Diluent RD5-3 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 reagents, standard dilutions, 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 RD1-47 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 MCP-5 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.

*Samples may require dilution. See Sample Preparation section.

CALCULATION OF RESULTS

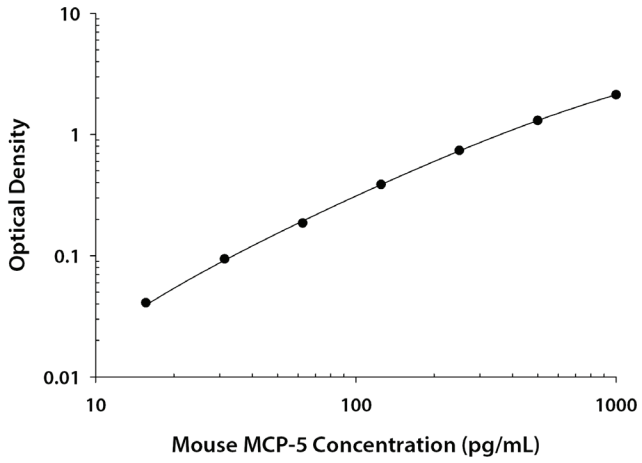
Average the duplicate readings for each standard, control, and sample and subtract the average zero standard optical density (O.D.).

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 MCP-5 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	0.020 0.025	0.022	—
15.6	0.063 0.063	0.063	0.041
31.3	0.116 0.117	0.116	0.094
62.5	0.203 0.214	0.208	0.186
125	0.405 0.413	0.409	0.387
250	0.750 0.770	0.760	0.738
500	1.320 1.327	1.324	1.302
1000	2.111 2.190	2.150	2.128

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)	32	85	318	31	86	318
Standard deviation	1.8	3.5	18	2.8	7.9	33
CV (%)	5.6	4.1	5.7	9.0	9.2	10.4

RECOVERY

The recovery of mouse MCP-5 spiked to three levels throughout the range of the assay was evaluated.

Sample Type	Average % Recovery	Range
Culture supernates (n=6)	94	81-119%
Serum* (n=6)	93	80-108%
EDTA plasma* (n=6)	99	84-114%

*Samples were diluted prior to assay as directed in the Sample Preparation section.

LINEARITY

To assess the linearity of the assay, samples containing and/or spiked with high concentrations of mouse MCP-5 were diluted with calibrator diluent to produce samples with values within the dynamic range of the assay.

		Cell culture supernates (n=6)	Serum* (n=6)	EDTA plasma* (n=6)
1:2	Average % of Expected	99	103	104
	Range (%)	91-110	99-105	100-109
1:4	Average % of Expected	100	105	110
	Range (%)	81-120	100-108	104-114
1:8	Average % of Expected	99	107	111
	Range (%)	81-120	105-112	101-117
1:16	Average % of Expected	100	113	111
	Range (%)	90-110	104-119	98-119

*Samples were diluted prior to assay as directed in the Sample Preparation section.

SENSITIVITY

Two assays were evaluated and the minimum detectable dose (MDD) of mouse MCP-5 ranged from 1.07-2.10 pg/mL. The mean MDD was 1.58 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 MCP-5 produced at R&D Systems®.

SAMPLE VALUES

Serum/Plasma - Samples were evaluated for detectable levels of mouse MCP-5 in this assay.

Sample Type	Mean (pg/mL)	Range (pg/mL)
Serum (n=20)	150	58-302
EDTA plasma (n=20)	158	50-674

Cell Culture Supernates:
Mouse splenocytes (1 x 10^6 cells/mL) were cultured for 3 days in RPMI plus 10% fetal bovine serum supplemented with 50 µM β-mercaptoethanol and 10 ng/mL recombinant human IL-2. An aliquot of the cell culture supernate was removed, assayed for mouse MCP-5, and measured 319 pg/mL.

Mouse lung conditioned media (2 lungs, 1-2 mm pieces in 40 mL of medium) was collected after culturing for 6 days. An aliquot of the cell culture supernate was removed, assayed for mouse MCP-5, and measured 314 pg/mL.

SPECIFICITY

This assay recognizes natural and recombinant mouse MCP-5.

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 MCP-5 control were assayed for interference. No significant cross-reactivity or interference was observed.

Recombinant mouse:

C10
Eotaxin
Fas Ligand
Flt-3 Ligand
G-CSF
GM-CSF
IFN-γ
IL-1α
IL-1β
IL-1ra
IL-2
IL-3
IL-4
IL-5
IL-6
IL-7
IL-9
IL-10
IL-10 R
IL-12 p40
IL-12 p70
IL-13
IL-17
IL-18
JE/MCP-1
KC
LIF
MARC
M-CSF
MIP-1α
MIP-1β
MIP-2
OPG
OSM
PlGF-2
RANTES
SCF
TARC
TNF-α
TNF RI
TNF RII
Tpo
RANK
RANK Ligand
VEGF
VEGF R1

Recombinant human:

MCP-1
MCP-2
MCP-3
MCP-4

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