Quantikine® ELISA

Human MDC Immunoassay

Catalog Number DMD00

For the quantitative determination of human Macrophage-Derived Chemokine (MDC) concentrations in cell culture supernate, 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

Macrophage-derived Chemokine (MDC) is a CC chemokine that is a potent chemoattractant for dendritic cells, natural killer cells, and the Th2 subset of peripheral blood T lymphocytes (1-3). In addition, MDC has also been shown to possess anti-HIV-1 activity (4-6). Expression of MDC mRNA has been identified in macrophages and dendritic cells (almost exclusively in the thymus) (1, 3). Immunohistochemical localization of MDC in normal human thymus revealed that most MDC was associated with medullary epithelial cells, with none detected in macrophages and dendritic cells (7). This apparent inconsistency may be related to phenotypically different populations of macrophages and dendritic cells. MDC at low concentrations (*i.e.* 1 ng/mL) is a chemoattractant for monocyte-derived dendritic cells, activated NK cells and activated T cells (1). Monocytes and a subpopulation of thymocytes migrate in response to much higher concentrations of MDC (*i.e.* 100 ng/mL) (1).

Dendritic cells, B lymphocytes, and macrophages all produce MDC constitutively, while NK cells, monocytes, and $CD4^+$ T lymphocytes produce MDC upon stimulation. The Th2 cytokines IL-4 and IL-13 are potent stimulators of monocyte MDC production, whereas IL-10 inhibits secretion of MDC (8, 9). IFN-γ can also suppress MDC expression in monocytes, macrophages, and dendritic cells (9).

MDC is a 69 amino acid, 8 kDa CC chemokine originally cloned from macrophage cDNA (1, 3). It shares less than 35% sequence identity with other human chemokines, with TARC (thymus- and activation-regulated chemokine) being its closest known human relative (3). The human chemokines MDC, fractalkine, and TARC are all clustered on chromosome 16q13 (10). MDC has 65% identity with the apparent mouse homologue, ABCD-1 (Activated B cell and dendritic cell-1) (11).

The biological actions of MDC are partially mediated through the G protein-coupled chemokine receptor, CCR4 (12). CCR4 is expressed on the Th2 subset of mature $CD4^+$ T lymphocytes (13, 14). Antigen-presenting cells may specifically recruit Th2 cells expressing CCR4 by producing MDC (15). Activation of CCR4 leads to a Ca^{2+} flux that can be used, in addition to chemotaxis, to bioassay MDC. Additional receptors may exist for MDC. Macrophages and dendritic cells express little or no CCR4, yet still respond to MDC by chemotaxis, thus suggesting the expression of an alternate receptor (1). MDC also induces human eosinophil chemotaxis in a CCR3 and CCR4-independent manner (16). MDC activates eosinophil chemotaxis, but does not elicit a measurable Ca^{2+} flux during this response.

The Quantikine® Human MDC Immunoassay is a 4.5 hour solid-phase ELISA designed to measure human MDC in cell culture supernates, serum, or plasma. It contains *E. coli*-expressed recombinant human MDC and has been shown to accurately quantitate the recombinant factor. Results obtained using naturally expressed human MDC 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 human MDC.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for human MDC has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any MDC present is bound by the immobilized antibody. After washing away any unbound substances, an enzyme-linked monoclonal antibody specific for human MDC is added to the wells. Following a wash to remove any unbound antibody-enzyme reagent, a substrate solution is added to the wells and color develops in proportion to the amount of MDC bound in the initial step. The color development is stopped and the intensity of the color is measured.

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.
- To ensure accurate results, proper adhesion of plate sealers during incubation steps is necessary.
- When using an automated plate washer, adding a 30 second soak period following the addition of Wash Buffer, and/or rotating the plate 180 degrees between wash steps may improve assay precision.
- 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. Wells that are green in color indicate that the Stop Solution has not mixed thoroughly with the Substrate 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
Human MDC Microplate	890780	96 well polystyrene microplate (12 strips of 8 wells) coated with a monoclonal antibody specific for human MDC.	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.*
Human MDC Conjugate	890778	21 mL of a monoclonal antibody specific for human MDC conjugated to horseradish peroxidase with preservatives.	May be stored for up to 1 month at 2-8 °C.*
Human MDC Standard	890779	Recombinant human MDC in a buffered protein base with preservatives; lyophilized. *Refer to the vial label for reconstitution volume.*	
Assay Diluent RD1-45	895146	11 mL of a buffered protein base with preservatives.	
Calibrator Diluent RD6-21	895261	21 mL of a buffered animal serum 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	895032	6 mL of 2 N sulfuric 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.
- 2-8 °C incubator or refrigerator
- 500 mL graduated cylinder.
- Test tubes for dilution of standards.
- Human MDC Controls (optional; R&D Systems®, Catalog # QC21).

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 and assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

Serum - Use a serum separator tube (SST) and allow samples to clot for 30 minutes at room temperature before centrifugation for 15 minutes at 1000 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 or heparin as an anticoagulant. Centrifuge for 15 minutes at 1000 x g within 30 minutes of collection. Assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

Note: *Citrate plasma has not been validated for use in this assay.*
Lipemic samples are not suitable for use in this assay.

REAGENT PREPARATION

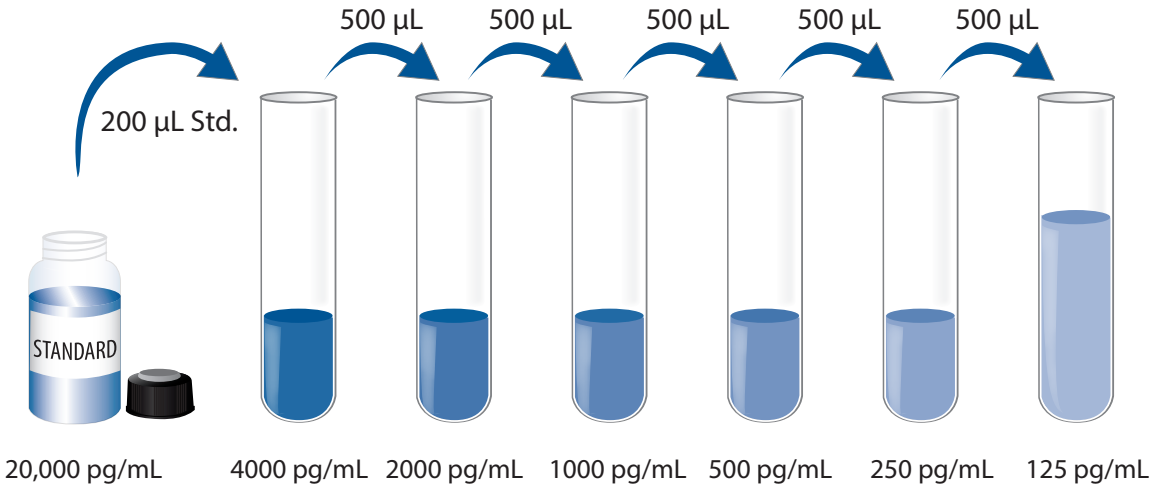
Bring all reagents to room temperature before use.

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. 200 µL of the resultant mixture is required per well.

Human MDC Standard - **Refer to the vial label for reconstitution volume.** Reconstitute the Human MDC Standard with deionized or distilled water. This reconstitution produces a stock solution of 20,000 pg/mL. Allow the standard to sit for a minimum of 15 minutes with gentle agitation prior to making dilutions.

Pipette 800 µL of Calibrator Diluent RD6-21 into the 4000 pg/mL tube. Pipette 500 µL into the remaining tubes. Use the stock solution to produce a dilution series (below). Mix each tube thoroughly before the next transfer. The 4000 pg/mL standard serves as the high standard. Calibrator Diluent RD6-21 serves as the zero standard (0 pg/mL).



ASSAY PROCEDURE

It is recommended that all standards, controls, and samples be assayed in duplicate.

1. Prepare all reagents and working standards 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 100 µL of Assay Diluent RD1-45 to each well.
4. Add 100 µL of standard or sample per well. Cover with the adhesive strip provided. Incubate for 2 hours **at 2-8 °C.**
5. Aspirate each well and wash, repeating the process three times for a total of four 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 200 µL of Human MDC Conjugate to each well. Cover with a new adhesive strip. Incubate for 2 hours **at 2-8 °C.**
7. Repeat the aspiration/wash as in step 5.
8. Add 200 µL of Substrate Solution to each well. Incubate for 30 minutes **at room temperature. Protect from light.**
9. Add 50 µL of Stop Solution to each well. The color in the wells should change from blue to yellow. If the color in the wells is green or the color change does not appear uniform, 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

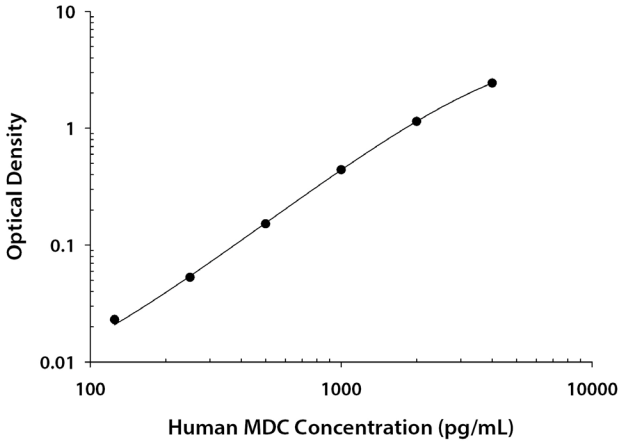
Average the duplicate readings for each standard, control, and sample and subtract the average zero standard optical density (O.D.).

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 human MDC 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	0.050 0.050	0.050	—
125	0.073 0.073	0.073	0.023
250	0.104 0.102	0.103	0.053
500	0.203 0.202	0.202	0.152
1000	0.488 0.494	0.491	0.441
2000	1.198 1.189	1.194	1.144
4000	2.505 2.467	2.486	2.436

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 forty 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	40	40	40
Mean (pg/mL)	284	452	821	276	440	810
Standard deviation	12.0	9.7	24.0	22.2	27.8	42.8
CV (%)	4.2	2.1	2.9	8.0	6.3	5.3

RECOVERY

The recovery of human MDC spiked to levels throughout the range of the assay was evaluated.

Sample Type	Average % Recovery	Range
Cell culture media (n=4)	98	90-110%

LINEARITY

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

		Cell culture media (n=4)	Serum (n=4)	EDTA plasma (n=4)	Heparin plasma (n=4)
1:2	Average % of Expected	99	100	102	102
	Range (%)	95-101	99-101	99-103	96-104
1:4	Average % of Expected	98	102	104	106
	Range (%)	94-104	98-105	98-107	102-112
1:8	Average % of Expected	97	101	107	107
	Range (%)	90-104	94-105	97-114	102-111

SENSITIVITY

The minimum detectable dose (MDD) of human MDC is typically less than 62.5 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 highly purified *E. coli*-expressed recombinant human MDC produced at R&D Systems®.

SAMPLE VALUES

Serum/Plasma - Samples from apparently healthy volunteers were evaluated for the presence of human MDC in this assay. No medical histories were available for the donors used in this study.

Sample Type	Mean (pg/mL)	Range (pg/mL)	Standard Deviation (pg/mL)
Serum (n=62)	1109	486-2932	372
EDTA plasma (n=32)	885	448-1906	315
Heparin plasma (n=32)	789	365-1837	300

Cell Culture Supernates - Human peripheral blood mononuclear cells (5 x 10^6 cells/mL) were cultured in RPMI supplemented with 5% fetal bovine serum, 50 µM β-mercaptoethanol, 2 mM L-glutamine, 100 U/mL penicillin, and 100 µg/mL streptomycin sulfate. The cells were cultured unstimulated or stimulated with 10 µg/mL PHA. Aliquots of the cell culture supernates were removed and assayed for levels of human MDC.

Condition	Day 1 (ng/mL)	Day 5 (ng/mL)
Unstimulated	10,810	17,760
Stimulated	4993	7358

SPECIFICITY

This assay recognizes natural and recombinant human MDC.

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 recombinant human MDC control were assayed for interference. No significant cross-reactivity or interference was observed.

Recombinant human:
GROα
GROβ
GROγ
MCP-1
MIP-1α
MIP-1β
RANTES

Recombinant mouse:
MDC
MIP-1α
MIP-1β

Recombinant porcine:
IL-8

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