BD OptEIA™

Human MCP-1 ELISA Kit

Instruction Manual

Catalog No. 559017

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Introduction

Human monocyte chemoattractant protein-1 (MCP-1), previously known as MCAF (monocyte chemotactic and activating factor), is a member of the CC chemokine family. MCP-1 is produced by a variety of stimulated cell types including monocytes, lymphocytes, endothelial cells and fibroblasts. MCP-1 is a potent chemoattractant for monocytes and it also activates lymphocytes, basophils and NK cells.

The BD OptEIA™ Human MCP-1 ELISA Kit is for the quantitative determination of human MCP-1 in serum, plasma, and cell culture supernatant.

Principle of the Test

The BD OptEIA test is a solid phase sandwich ELISA (Enzyme-Linked Immunosorbent Assay). It utilizes a monoclonal antibody specific for MCP-1 coated on a 96-well plate. Standards and samples are added to the wells, and any MCP-1 present binds to the immobilized antibody. The wells are washed and streptavidin-horseradish peroxidase conjugate mixed with biotinylated anti-mouse antibody is added, producing an antibody-antigen-antibody "sandwich". The wells are again washed and TMB substrate solution is added, which produces a blue color in direct proportion to the amount of MCP-1 present in the initial sample. The Stop Solution changes the color from blue to yellow, and the microwell absorbances are read at 450 nm.

Reagents Provided

Antibody Coated Wells:	2 plates of 96 breakable wells (12 strips × 8 wells) coated with anti-human MCP-1 monoclonal antibody
Detection Antibody:	30 mL of biotinylated anti-human MCP-1 monoclonal antibody with 0.015% ProClin™- 150 as preservative
Standards:	4 vials lyophilized recombinant human MCP-1
Enzyme Concentrate (250×):	150 µL of 250× concentrated Streptavidin-horseradish peroxidase conjugate with BSA* and ProClin™- 150 as preservative
Standard Diluent:	30 mL of animal serum* base with 0.15% ProClin™-150 as preservative
Wash Concentrate (20×):	100 mL of 20× concentrated detergent solution with ProClin™- 150 as preservative
Substrate Reagent A:	13 mL of hydrogen peroxide in buffered solution
Substrate Reagent B:	30 mL of 3,3',5,5'-tetramethylbenzidine (TMB) in buffered solution
Stop Solution:	13 mL of 1 M phosphoric acid
Plate Sealers:	4 sheets with adhesive backing

**Source of all serum proteins is from USDA inspected abattoirs located in the United States*

Materials Required but not Provided

- Microplate reader capable of measuring absorbance at 450 nm
- Precision pipettes to deliver 50 µL and 100 µL volumes
- Adjustable 1 mL, 5 mL, 10 mL, 25 mL pipettes for reagent preparation
- Deionized or distilled water
- Wash bottle or automated microplate washer
- Log-log graph paper or computer and software for ELISA data analysis
- Tubes to prepare standard dilutions
- Laboratory timer
- Absorbent paper

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Storage Information

1. Store kit at 2 - 8°C. Do not use kit after expiration date.
2. Before use, bring all reagents to room temperature (18 - 25°C). Immediately after use, return to proper storage conditions.
3. Lyophilized standards are stable until kit expiration date. After reconstitution, use freshly reconstituted standard within 12 hours (stored at 2 - 8°C).

Warnings and Precautions

1. Reagents that contain preservatives may be toxic if ingested, inhaled, or brought in contact with skin.
2. Avoid contact of skin, eyes, or clothing with Stop Solution or Substrate Reagents.
3. Handle all serum and plasma specimens in accordance with NCCLS guidelines for preventing transmission of blood-borne infections.
4. ***Warning***

   Wash Concentrate (20X) (component 51-9003738) contains 0.002% (w/w), Human MCP-1 Lyophilized Standard (component 51-26596E) contains 0.03% (w/w), Standard Diluent (component 51-2604KC) contains 0.003% (w/w) and Detection Antibody Biotin Anti-Human MCP-1 (component 51-26592E) contains 0.003% (w/w) of a CMIT/MIT mixture (3:1), which is a mixture of: 5-chloro-2-methyl-4-isothiazolin-3-one [EC No 247-500-7] and 2-methyl-4-isothiazolin-3-one [EC No 220-239-6] (3:1).

   Hazard statements

   May cause an allergic skin reaction.

   Precautionary statements

   Wear protective gloves / eye protection.

   Wear protective clothing.

   Avoid breathing mist/vapours/spray.

   If skin irritation or rash occurs: Get medical advice/attention.

   IF ON SKIN: Wash with plenty of water.

   Dispose of contents/container in accordance with local/regional/national/international regulations.

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5. ***Danger***

Stop Solution (component 51-2608KC) contains 15.23% phosphoric acid (w/w).

Hazard statements

Causes severe skin burns and eye damage.

Precautionary statements

Wear protective gloves / eye protection.

Wear protective clothing.

IF ON SKIN (or hair): Remove/Take off immediately all contaminated clothing. Rinse skin with water/shower.

IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do.

Continue rinsing.

IF INHALED: Remove victim to fresh air and keep at rest in a position comfortable for breathing.

Dispose of contents/container in accordance with local/regional/national/international regulations.

6. ***Danger***

Substrate Reagent B (component 51-2607KD) *contains 33.05% methanol (w/w).*

Hazard statements

Flammable liquid and vapor.

Toxic if swallowed, in contact with skin or if inhaled.

Causes damage to the central nervous system. Route of exposure: Oral.

Precautionary statements

Keep away from heat/sparks/open flames/hot surfaces. - No smoking.

Wear protective gloves / eye protection.

Wear protective clothing.

Do not breathe mist/vapours/spray.

IF ON SKIN (or hair): Remove/Take off immediately all contaminated clothing. Rinse skin with water/shower.

IF INHALED: Remove victim to fresh air and keep at rest in a position comfortable for breathing.

Specimen Collection and Handling

Specimens should be clear, non-hemolyzed and non-lipemic. Samples with expected values higher than the top standard, 1000 pg/mL, should be diluted with Standard Diluent prior to running the assay.

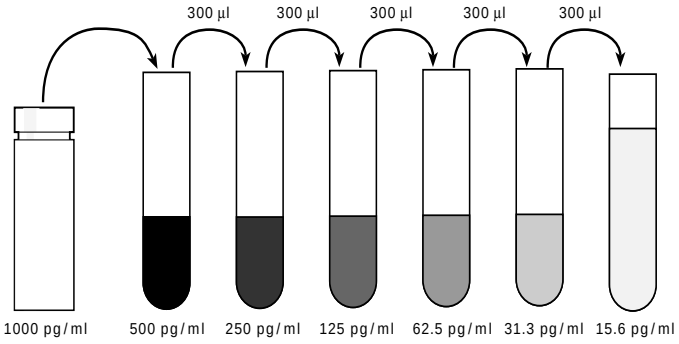
Cell culture supernatants: Remove any particulate material by centrifugation and assay immediately or store samples at ≤ -20°C. Avoid repeated freeze-thaw cycles.

Serum: Use a serum tube (eg, BD Vacutainer® Cat. No. 366430) and allow samples to clot for 30 minutes, then centrifuge for 10 minutes at 1000 × *g*. Remove serum and assay immediately or store samples at ≤ -20°C. Avoid repeated freeze-thaw cycles.

Plasma: Collect plasma using citrate, EDTA, or heparin as anticoagulant. Centrifuge for 10 minutes at 1000 × g within 30 minutes of collection. Assay immediately or store samples at ≤ -20°C. Avoid repeated freeze-thaw cycles.

Reagent Preparation

1. Bring all reagents to room temperature (18 - 25°C) before use.
2. Standards
   a. After warming to room temperature, carefully open vial to avoid loss of material. Reconstitute 1 vial lyophilized Standard with required volume (noted on vial label) of Standard/Sample Diluent to prepare a 1000 pg/mL stock standard. Allow the standard to equilibrate for at least 15 minutes before making dilutions. Gently vortex to mix.
   b. Add 300 µL Standard/Sample Diluent to 6 tubes. Label as 500 pg/mL, 250 pg/mL, 125 pg/mL, 62.5 pg/mL, 31.3 pg/mL, and 15.6 pg/mL.
   c. Perform serial dilutions by adding 300 µL of each standard to the next tube and vortexing between each transfer. The undiluted standard serves as the high standard (1000 pg/mL). The Standard/Sample Diluent serves as the zero standard (0 pg/mL).



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3. Working Detector

   Note: One-step incubation of Biotin/Streptavidin reagents. See ***Assay Procedure***, step 5.

4. Wash Buffer

   Note: If the Wash Concentrate contains visible crystals, warm to room temperature and mix gently until dissolved. Dilute required quantity of 20× Wash Concentrate with deionized or distilled water, mix. (To prepare 2,000 mL, add 100 mL Wash Concentrate to 1,900 mL water. At least 500 mL solution should be prepared for a full 96-well plate).

5. Substrate Solution

   Within 15 minutes prior to use, mix equal volumes of Substrate Reagent A and Substrate Reagent B in a clean glass tube or flask. Make only the amount required for each run (for a full 96-well plate, prepare 6 mL + 6 mL = 12 mL solution). Discard any remaining working solution after use. Avoid prolonged exposure to light or contact with metal, air, or extreme temperature as color may develop.

Assay Procedure

1. Bring all reagents and samples to room temperature (18 - 25°C) prior to use. It is recommended that all standards and samples be run in duplicate. A standard curve is required in each assay run.

2. Remove required quantity of test strips/wells, place in well holder.

   Note: Wells are provided in breakable 8-well strips. Strips may be "broken" into individual wells, replaced in well holder, and assayed. Return any unused wells to sealed pouch for 2 - 8°C storage.

3. Pipette 100 µL of each standard (see ***Reagent Preparation***, step 2) and sample into appropriate wells. Gently shake/tap the plate for 5 seconds to mix. Cover wells with Plate Sealer and incubate for 2 hours at room temperature.

4. Prepare Working Detector. Within 15 minutes prior to use, pipette required volume of Detection Antibody into a clean tube or flask. Add in required quantity of Enzyme Concentrate (250×), vortex or mix well. For a full 96-well plate, add 48 µL of Enzyme Concentrate into 12 mL of Detection Antibody.

5. Decant or aspirate contents of wells. Wash wells by filling with at least 300 µL/well prepared Wash Buffer (see ***Reagent Preparation***, step 4), followed by decanting/aspirating. Repeat wash 4 times for a total of 5 washes. After the last wash, blot plate on absorbent paper to remove any residual buffer. Complete removal of liquid is required for proper performance.

6. Add 100 µL of prepared Working Detector (see ***step* 4** above) to each well. Cover wells with Plate Sealer and incubate for 1 hour at room temperature.
7. Wash wells as in Step 5, but a total of 7 times.

   Note: In this final wash step, soak wells in wash buffer for 30 seconds to 1 minute for each wash. Thorough washing at this step is very important.
8. Add 100 µL of prepared Substrate Solution (see ***Reagent Preparation***, step 5) to each well. Incubate plate (without Plate Sealer) for 30 minutes at room temperature in the dark
9. Add 50 µL of Stop Solution to each well.
10. Read absorbance at 450 nm within 30 minutes of stopping reaction. If wavelength correction is available, subtract the optical density readings at 570 nm from readings at 450 nm.

Assay Procedure Summary

1. Add 100 µL standard or sample to each well.
   Incubate 2 hours at room temperature.
2. Aspirate and wash 5 times.
3. Add 100 µL prepared Working Detector to each well.
   Incubate 1 hour at room temperature.
4. Aspirate and wash/soak 7 times.
5. Add 100 µL Substrate Solution to each well.
   Incubate 30 minutes at room temperature.
6. Add 50 µL Stop Solution to each well.
   Read at 450 nm within 30 minutes.
   λ correction 570 nm.

Calculation of Results

Calculate the mean absorbance for each set of duplicate standards, controls and samples. Subtract the mean zero standard absorbance (ie, plate background) from each.

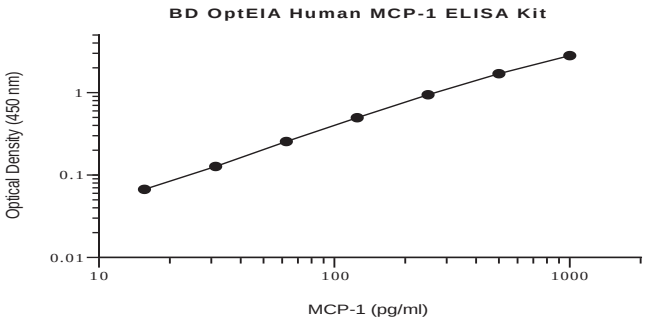
Plot the standard curve on log-log graph paper, with MCP-1 concentration on the x-axis and absorbance on the y-axis. Draw the best fit straight line through the standard points.

To determine the MCP-1 concentration of the unknowns, find the unknowns' mean absorbance value on the y-axis and draw a horizontal line to the standard curve. At the point of intersection, draw a vertical line to the x-axis and read the MCP-1 concentration. If samples were diluted, multiply the interpolated MCP-1 concentration by the dilution factor.

Computer-based curve-fitting statistical software may also be employed.

Typical Data

This standard curve is for demonstration only. A standard curve must be run with each assay.



Concentration (pg/mL)	OD1	OD2	Mean	Zero Standard Subtracted
0	0.044	0.052	0.048	0.000
15.6	0.116	0.115	0.116	0.067
31.3	0.174	0.177	0.176	0.127
62.5	0.301	0.305	0.303	0.255
125	0.545	0.543	0.544	0.496
250	1.001	0.980	0.991	0.942
500	1.738	1.752	1.745	1.697
1000	2.864	2.856	2.860	2.812

Limitations of the Procedure

1. Interference by drug metabolites, soluble receptors, or other binding proteins in specimens has not been thoroughly investigated. The possibility of interference cannot be excluded.
2. This kit is intended for use as an integral unit. Do not mix reagents from different kit lots. Reagents from other manufacturers/other available clones should not be used in this kit.

Performance

Limit of Detection

The minimum detectable dose of MCP-1 was determined to be 1.0 pg/mL. This is defined as two standard deviations above the mean optical density of 20 replicates of the zero standard.

Recovery

Various matrices were diluted 1:10 with Standard Diluent, then human MCP-1 was spiked in at three different levels within the assay range. Results are compared with same amounts of MCP-1 spiked in Standard Diluent alone, as follows:

	Observed in Standard Diluent (pg/mL)	Observed in given matrix (pg/mL)	% Recovery
Serum	907.2 224.9 53.7	881.8 226.0 82.6	97 100 154
Plasma	907.2 224.9 53.7	853.9 225.9 86.3	94 100 161
Cell culture supernatant	896.6 218.2 46.3	896.9 197.9 43.1	100 91 93

Linearity

The following matrices diluted 1:10 with Standard Diluent were spiked with MCP-1, then serially diluted with Standard Diluent.

	Dilution	Expected (pg/mL)	Observed (pg/mL)	% of Expected
Serum	undiluted	-	881.8	-
	1:2	440.9	493.6	112
	1:4	220.5	253.5	115
	1:8	110.2	125.9	114
	1:16	55.1	62.2	112
Plasma	undiluted	-	853.8	-
	1:2	426.9	447.8	105
	1:4	213.5	232.1	109
	1:8	106.7	116.7	110
	1:16	53.4	59.0	110
Cell culture supernatant	undiluted	-	896.8	-
	1:2	448.4	507.0	114
	1:4	224.2	259.0	115
	1:8	112.1	123.4	110
	1:16	56.0	60.6	109

Specificity

Cross Reactivity: The factors listed below were spiked in Standard Diluent at 100 ng/mL to test for any cross reactivity with the BD OptEIA Human MCP-1 ELISA assay. No cross reactivity was identified.

Interference: The factors listed below were spiked at 100 ng/mL in Standard Diluent with 100 pg/mL MCP-1 to test for any interference with the quantitation of human MCP-1. No effect on assay results was observed.

Recombinant Human

IL-1α, IL-1β, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-11, IL-12 (p40), IL-12 (p70), IL-13, IL-15, Eotaxin, IFN-γ, G-CSF (10 ng/mL), GM-CSF, GRO, CD23, Lymphotactin (10 ng/mL), MIP-1β, MCP-2, MCP-3, MCP-4, NAP2, IP-10, NT-3, PDGF-AA, SCF (10 ng/mL), TNF, Lt-α, VEGF

Recombinant Mouse

IL-1β, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-9, IL-10, IL-12, IL-15, IFN-γ, GM-CSF, MCP-1, MIG, TCA3, MCP-1

Recombinant Rat

IL-2, IL-4, IL-6, IL-10, GM-CSF, IFN-γ, MCP-1, RANTES

Other

Viral IL-10 (10 ng/mL)

Interfering Substances:

The following substances at levels ≥ 2 mg/mL were added to Standard Diluent spiked with 200 pg/mL MCP-1. No effect on assay results was observed.

- Bilirubin
- Human hemoglobin
- Human transferrin
- Triglycerides
- Heparin (300 units/mL)
- Sodium Citrate
- EDTA

Precision

Intra-assay

Twenty-four replicates each of three different levels of MCP-1 were tested in one plate. The following results were observed:

Number of Replicates (n)	24	24	24
Mean Concentration	780 pg/mL	279 pg/mL	80 pg/mL
SD	17.9	10.4	8.2
%CV	2.3	3.7	10.3

Inter-assay

Three different levels of MCP-1 were tested in four different plates. The following results were observed:

Number of Replicates (n)	32	32	32
Mean Concentration	810 pg/mL	245 pg/mL	63 pg/mL
SD	43.7	12.4	5.4
%CV	5.4	5.1	8.5

Standardization

This immunoassay is calibrated against recombinant human MCP-1.

Experimental Results

Serum/Plasma

Ten serum samples were tested in this assay. The mean value was 350 pg/mL, with a range from 160 to 769 pg/mL.

Twelve high-titer rheumatoid factor serum samples were tested in this assay. The mean value was 515 pg/mL, with a range from 155 to 1626 pg/mL.

Cell Culture Supernatants

Human peripheral blood mononuclear cells were cultured in RPMI 1640 complete medium with 10% fetal bovine serum at 1×10^6 cells/mL, and activated with TPA at 50 ng/mL and A23187 at 1 µg/mL. After 24 hours, culture supernatants were collected and quantitated for MCP-1 using a BD OptEIA Human MCP-1 ELISA Kit. The results are as follows:

Donor No.	MCP-1 (pg/mL)
1	70
2	8900
3	2400

Troubleshooting

Problem	Possible Source	Corrective Action
Poor Precision	• Inadequate washing / aspiration of wells • Inadequate mixing of reagents • Imprecise / inaccurate pipetting • Imprecise sealing of plate	• Check function of washing system • Ensure adequate mixing • Check / calibrate pipettes • Ensure complete sealing of plate
Poor Standard Curve	• Improper standard handling / dilution • Incomplete washing / aspiration of wells • Imprecise / inaccurate pipetting	• Ensure correct preparation of standards • Check function of washing system • Check / calibrate pipettes
Low Signal	• Inadequate reagent volumes added to wells • Incorrect incubation times / temperature • Overly high wash / aspiration pressure from automated plate-washer.	• Check / calibrate pipettes • Ensure sufficient incubation times / reagents warmed to room temperature • Utilize manual washing

References

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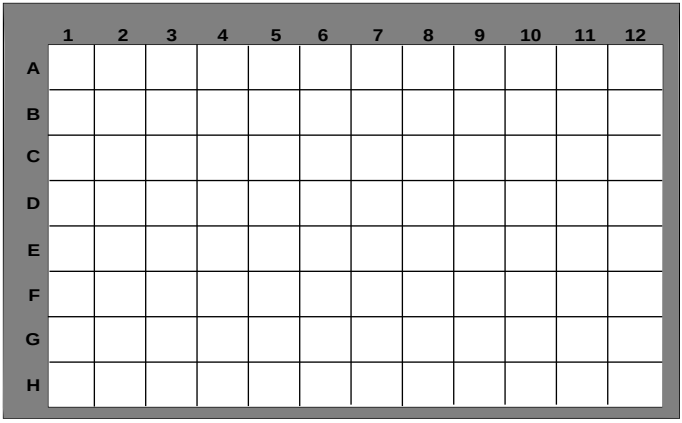
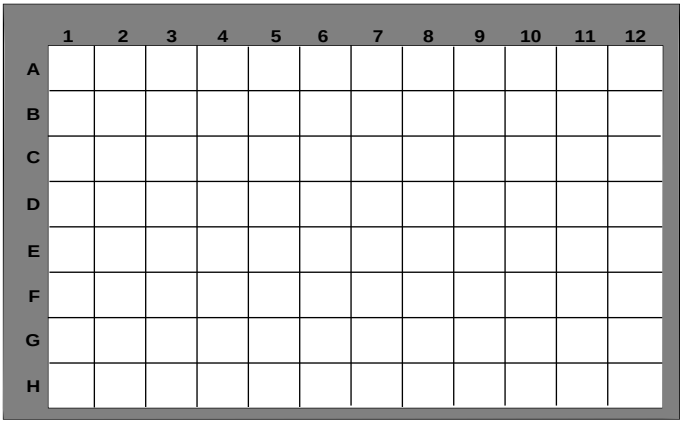
Plate Templates





Notes

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