Human MCP-1 Conferma™ ELISA

96-Well Plate Assay

Cat. # EZMCP1-99KRM (EZMCP1-99K5PKRM, EZMCP1-99K10PKRM)

HUMAN MCP-1 CONFERMA™ ELISA KIT
96-Well Plate

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MilliporeSigma.com

INTENDED USE

This Human MCP-1 Conferma™ ELISA kit is used for the non-radioactive quantification of Human MCP-1 in serum and plasma samples. One kit is sufficient to measure 36 unknown samples in duplicate. ***This kit is for Research Use Only. Not for Use in Diagnostic Procedures.***

PRINCIPLES OF ASSAY

This assay is a Sandwich ELISA that uses in-house developed critical reagents, including the Monoclonal Antibodies (mAb) and calibration material, to detect endogenous MCP-1 in biological fluids such as Human serum or plasma.

The Sandwich ELISA first binds MCP-1 using a specific capture Mouse anti-human MCP-1 monoclonal antibody bound to the wells of a 96 well microtitre plate. Following the addition of the sample, the assay is incubated for two hours, during which time endogenous or recombinant antigen (depending on the well) is bound by the mAb. The unbound material is washed off post-incubation, and a biotinylated mouse anti-human MCP-1 monoclonal antibody is added to complete the “Sandwich.” After an incubation period, the unbound material is washed off. The next step is a final incubation step, during which a streptavidin-horseradish peroxidase conjugate binds to the immobilized biotinylated antibodies. Following a final wash, horseradish peroxidase substrate, 3,3’,5,5’-tetramethylbenzidine is added. The enzyme activity is measured spectrophotometrically by the increased absorbance at 450-590 nm after acidification of formed products by addition of Stop Solution. The increase in absorbance is directly proportional to the amount of captured Human MCP-1. Quantitation of the analyte is derived by interpolation from a reference curve comprised of standard points of known concentrations of recombinant human MCP-1.

REAGENTS SUPPLIED

Each kit is sufficient to run one 96-well plate and contains the following reagents:

Note: Store all reagents at 2-8°C

Reagents Supplied	Catalog Number	Volume	Quantity
Human MCP-1 Conferma™ ELISA plate with 2 sealers	EP99	---------	1 plate 2 sealers
Human MCP-1 Standard	E8099-K	lyophilized	1 vial
Human MCP-1 Quality Controls 1, 2 and 3	E6099-1-K E6099-2-K E6099-3-K	lyophilized	1 vial each
Standard Diluent	SD-099	12 mL	1 vial
Assay Buffer	EAB099	10 mL	1 vial
10X HRP Wash Buffer for ELISA	EWB-HRP99	50 mL	2 bottles
Human MCP-1 Detection Antibody	E1099	12 mL	1 bottle
Enzyme Solution (100X)	EHRP-99	150 µL	1 bottle
Enzyme Solution Diluent	ED-099	12 mL	1 bottle
Substrate	ESS-TMB99	12 mL	1 bottle
Stop Solution	ET-TMB99	12 mL	1 bottle

STORAGE AND STABILITY

Recommended storage for kit components is 2-8°C.

All components are shipped and stored at 2-8°C. Reconstituted standards and controls can be frozen for future use, but repeated freeze/thaw cycles should be avoided.

10X Wash Buffer does not contain a preservative. After dilution, the 1X Wash Buffer may be filter sterilized (Stericup® filter, Millipore Sigma- Cat# SCGPU11RE) for storage of up to 1 month at 2 - 8°C. If not filter sterilized, all remaining 1X wash buffer should not be used after one week.

Refer to expiration dates on all reagents prior to use. Do not mix reagents from different kits unless they have the same lot numbers.

REAGENT PRECAUTIONS

Sodium Azide has been added to some reagents as a preservative. Although the concentration is low, Sodium Azide may react with lead and copper plumbing to form highly explosive metal azides. Dispose of unused contents and waste in accordance with international, federal, state, and local regulations.

Full Hazard label:

Ingredient, Cat #		Full Label	
Human MCP-1 Standard	E8099-K		**Danger:** Harmful if swallowed or if inhaled. Toxic in contact with skin. Causes serious eye damage. May cause damage to the brain through prolonged or repeated exposure. Do not breathe dust/ fume/ gas/ mist/ vapors/ spray. Wash skin thoroughly after handling. Do not eat, drink, or smoke when using this product. Use only outdoors or in a well-ventilated area. Wear protective gloves/ eye protection/ face protection. IF SWALLOWED: Call a POISON CENTER/ doctor if you feel unwell. IF ON SKIN: Wash with plenty of soap and water. IF INHALED: Remove person to fresh air and keep comfortable for breathing. IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present, and easy to do. Continue rinsing. Immediately call a POISON CENTER/ doctor. Specific measures (see supplemental first aid instructions on this label). Rinse mouth. Remove/ Take off immediately all contaminated clothing. Wash contaminated clothing before reuse. Store locked up. Dispose of contents/ container to an approved waste disposal plant.

Human MCP-1 Quality Control 1, 2 & 3	E6099-1-K E6099-2-K E6099-3-K		**Danger**: Harmful if swallowed or if inhaled. Toxic in contact with skin. Causes serious eye damage. May cause damage to the brain through prolonged or repeated exposure. Do not breathe dust/ fume/ gas/ mist/ vapors/ spray. Wash skin thoroughly after handling. Do not eat, drink, or smoke when using this product. Use only outdoors or in a well-ventilated area. Wear protective gloves/ eye protection/ face protection. IF SWALLOWED: Call a POISON CENTER/ doctor if you feel unwell. IF ON SKIN: Wash with plenty of soap and water. IF INHALED: Remove person to fresh air and keep comfortable for breathing. IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present, and easy to do. Continue rinsing. Immediately call a POISON CENTER/ doctor. Specific measures (see supplemental first aid instructions on this label). Rinse mouth. Remove/ Take off immediately all contaminated clothing. Wash contaminated clothing before reuse. Store locked up. Dispose of contents/ container to an approved waste disposal plant.
Human MCP-1 detection antibody	E1099		**Warning**: Causes serious eye irritation. May cause damage to organs Respiratory Tract through prolonged or repeated exposure. Do not breathe dust/ fume/ gas/ mist/ vapors/ spray. Wash skin thoroughly after handling. Wear eye protection/ face protection. IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present, and easy to do. Continue rinsing. Get medical advice/ attention if you feel unwell. If eye irritation persists: Get medical advice/ attention. Dispose of contents/ container to an approved waste disposal plant.

Standard Diluent	SD-099		**Warning:** Causes serious eye irritation. May cause damage to organs Respiratory Tract through prolonged or repeated exposure. Do not breathe dust/ fume/ gas/ mist/ vapors/ spray. Wash skin thoroughly after handling. Wear eye protection/ face protection. IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present, and easy to do. Continue rinsing. Get medical advice/ attention if you feel unwell. If eye irritation persists: Get medical advice/ attention. Dispose of contents/ container to an approved waste disposal plant.
Assay Buffer	EAB099		**Warning:** Causes serious eye irritation. May cause damage to organs Respiratory Tract through prolonged or repeated exposure. Do not breathe dust/ fume/ gas/ mist/ vapors/ spray. Wash skin thoroughly after handling. Wear eye protection/ face protection. IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present, and easy to do. Continue rinsing. Get medical advice/ attention if you feel unwell. If eye irritation persists: Get medical advice/ attention. Dispose of contents/ container to an approved waste disposal plant.
Enzyme Solution (100X)	EHRP-99		**Warning:** May cause an allergic skin reaction. Wear protective gloves. IF ON SKIN: Wash with plenty of soap and water. Wear protective gloves. IF ON SKIN: Wash with plenty of soap and water.
Enzyme Solution Diluent	ED-099		**Warning:** May cause an allergic skin reaction. Wear protective gloves. IF ON SKIN: Wash with plenty of soap and water.

Stop Solution	ET-TMB99		**Warning:** May be corrosive to metals.

MATERIALS REQUIRED BUT NOT PROVIDED

1. Multi-channel Pipettes and pipette tips: 5-50 µL and 50-300 µL
2. Pipettes and pipette tips: 10 µL-20 µL or 20 µL-100 µL
3. Reagent Reservoirs
4. Polypropylene Microfuge Tubes
5. Vortex Mixer
6. De-ionized water
7. Microtiter Plate Reader capable of reading absorbency at 450 nm
8. Orbital Microtiter Plate Shaker
9. Absorbent Paper or Cloth

SAMPLE COLLECTION AND STORAGE

A. Preparation of Serum Samples:

- Allow the blood to clot for at least 30 minutes before centrifugation for 10 minutes at 1000xg. Remove serum and assay immediately or aliquot and store samples at ≤ -20°C.
- Avoid multiple >2 freeze/thaw cycles.
- When using frozen samples, it is recommended to thaw the samples completely, mix well by vortexing, and centrifuge prior to use in the assay to remove particulates.
- Serum samples should be used 1:5 diluted in the standard diluent provided in the kit. For example, in a tube, 30 µL of serum may be combined with 120 µL of standard diluent.

B. Preparation of Plasma Samples:

- Plasma collection using EDTA as an anticoagulant is recommended. Centrifuge for 10 minutes at 1000xg within 30 minutes of blood collection. Remove plasma and assay immediately or aliquot and store samples at ≤ -20°C.
- Avoid multiple >2 freeze/thaw cycles.
- When using frozen samples, it is recommended to thaw the samples completely, mix well by vortexing and centrifuge prior to use in the assay to remove particulates.
- Plasma samples should be used 1:5 diluted in the standard diluent provided in the kit. For example, in a tube, 30 µL of plasma may be combined with 120 µL of standard diluent.

C. Preparation of Tissue Culture Supernatant:

- Centrifuge the sample to remove debris and assay immediately or aliquot and store samples at ≤ -20°C.
- Avoid multiple (>2) freeze/thaw cycles.
- Tissue culture supernatant may require dilution with an appropriate control medium prior to assay. Tissue/cell extracts should be done in neutral buffers containing reagents and conditions that do not interfere with assay performance. Excess concentrations of detergent, salt, denaturants, high or low pH, etc. will negatively affect the assay. Organic solvents should be avoided. The tissue/cell extract samples should be free of particles such as cells or tissue debris.

NOTE:

- A maximum of 50 µL per well of diluted serum or plasma can be used. Tissue culture or other media may also be used.
- All samples must be stored in polypropylene tubes. **DO NOT STORE SAMPLES IN GLASS.**
- Avoid debris, lipids, and cells when using samples with gross hemolysis or lipemia.
- Care must be taken when using heparin as an anti-coagulant since an excess of heparin will provide falsely high values. Use no more than 10 IU heparin per mL of blood collected.

REAGENT PREPARATION

A. Human MCP-1 Standard Preparation

Use care in opening the lyophilized Standard vial. Refer to the Standard reconstitution instructions provided on the Certificate of analysis to hydrate the stock standard vial to 1X concentration

2. For dilution series, Label 7 polypropylene microfuge tubes as Std 7, Std 6, Std 5, Std 4, Std 3, Std 2 and Std 1. Add 200 µL of Standard diluent to each of the 7 tubes. Prepare serial dilutions by adding 200 µL of the reconstituted standard to the Std 7 tube, mix well and transfer 200 µL of the of the Std 7 to the Std 6 tube, mix well and transfer 200 µL of the Std 6 to the Std 5 tube, mix well and transfer 200 µL of the Std 5 to the Std 4 tube, mix well and transfer 200 µL of the Std 4 to the Std 3 tube, mix well and transfer 200 µL of the Std 3 to the Std 2 tube, mix well and transfer 200 µL of the Std 2 to the Std 1 tube and mix well. The 0 pg/mL standard (Background) will be Standard diluent.
   Note: Change tip for every dilution. Wet tip with the standard before dispensing. Unused portions of the reconstituted standard should be stored in small aliquots at ≤ -20°C. Avoid multiple freeze/thaw cycles.

Tube #	Volume of Deionized Water to Add	Volume of Standard Diluent to Add	Standard Stock Concentration
Reconstituted standard	Refer to COA	Refer to COA	1000 pg/mL

Tube #	Volume of Standard Diluent to Add	Volume of Standard to Add	Standard Concentration pg/mL
Standard 7	200 µL	200 µL of reconstituted standard	500
Standard 6	200 µL	200 µL of Standard 7	250
Standard 5	200 µL	200 µL of Standard 6	125
Standard 4	200 µL	200 µL of Standard 5	62.5
Standard 3	200 µL	200 µL of Standard 4	31.2
Standard 2	200 µL	200 µL of Standard 3	15.6
Standard 1	200 µL	200 µL of Standard 2	7.8

B. Human MCP-1 Quality Control 1, 2 and 3 Preparation

Use care in opening the lyophilized Quality Control vials. Reconstitute each Human MCP-1 Quality Control 1, 2, and 3 as per the instructions provided in the Certificate of Analysis. Once hydrated, controls can be stored in small aliquots at ≤ -20°C. Avoid further freeze/thaw cycles.

REAGENT PREPARATION (continued)

C. Preparation of Wash Buffer

Bring the 10X Wash Buffer to room temperature and mix to bring all salts into solution. Dilute 100 mL of 10X Wash Buffer (two bottles) with 900 mL deionized water.

NOTE: 10X Wash Buffer does not contain a preservative. For storage of up to 1 month at 2 - 8°C, the 1X Wash Buffer may need to be filter sterilized (Stericup® filter, Millipore Sigma- Cat# SCGPU11RE)

D. Preparation of Enzyme Solution

Add 120 µL of 100X enzyme solution to the bottle containing 12 mL of enzyme solution diluent. Mix well. Store unused portion at 2-8°C for up to one month.

Human MCP-1 Conferma™ ELISA ASSAY PROCEDURE

Warm all reagents to room temperature before setting up the assay.

1. Remove the required number of strips from the Microtiter Assay Plate. Unused strips should be resealed in the foil pouch and stored at 2-8°C. Assemble the strips in an empty plate holder. Add 300 µL diluted Wash Buffer to each well of the plate. Decant Wash Buffer and remove the residual volume by inverting the plate and tapping it smartly onto absorbent towels several times. **Do not let wells dry before proceeding to the next step.** If an automated machine is used for the assay, follow the manufacturer's instructions for all washing steps described in this protocol.

2. Add 50 µL of appropriate assay buffer to Blank, Standards, Quality Control, and sample wells (refer to Microtiter Plate Arrangement section for suggested sample order placement). When assaying serum or plasma, use EAB099**.** When assaying tissue culture or other supernatants, use proper control culture medium as the assay buffer.

3. Add 50 µL Standard diluent to the Blank wells.

4. Add 50 µL of Standards or Controls to the appropriate wells.

5. Add 50 µL of diluted sample to the appropriate wells.

6. Cover the plate with a plate sealer and incubate at room temperature for 2 hours on an orbital microtiter plate shaker set to rotate at moderate speed, about 400 to 500 rpm.

7. Remove plate sealer and decant reagents from the plate. Tap as before to remove residual volume in well. Wash wells 3 times with diluted Wash Buffer, 300 µL per well per wash. Decant and tap after each wash to remove residual buffer.

Human MCP-1 Conferma™ ELISA ASSAY PROCEDURE (continued)

8. Add 100 µL Detection Antibody to each well. Re-cover plate with sealer and incubate at room temperature for 1 hour on an orbital microtiter plate shaker set to rotate at moderate speed, approximately 400 rpm.

9. Remove plate sealer and decant reagents from the plate. Tap as before to remove residual volume in well. Wash wells 3 times with diluted Wash Buffer, 300 µL per well per wash. Decant and tap after each wash to remove residual buffer.

10. Add 100 µL of 1X Enzyme Solution to each well. Cover the plate with sealer and incubate with moderate shaking at room temperature for 30 minutes on the microtiter plate shaker.

11. Remove sealer, decant reagents from the plate, and tap the plate to remove the residual volume. Wash wells 5 times with diluted Wash Buffer, 300 µL per well per wash. Decant and tap after each wash to remove residual buffer.

12. Add 100 µL of Substrate Solution to each well, cover plate with sealer, and shake on the plate shaker for approximately 15-25 minutes. Blue color should be formed in wells of the MCP-1 standards with intensity proportional to increasing concentrations of MCP-1.

    Note: Please be aware that the color may develop more quickly or more slowly than the recommended incubation time, depending on the localized room temperature. Please visually monitor the color development to optimize the incubation time.

13. Remove sealer and add 100 µL Stop Solution **[CAUTION: CORROSIVE SOLUTION]** and gently shake plate by hand to ensure complete mixing of the solution in all wells. The blue color should turn to yellow after acidification. Wipe the bottom of the microtiter plate to remove any residue prior to reading on a plate reader. Read absorbance at 450 nm and 590 nm in a plate reader within 5 minutes and ensure that there are no air bubbles in any well. Record the difference in absorbance units. The absorbance of the highest MCP-1 standard should be approximately 2.0 - 3.0, or not to exceed the capability of the plate reader used.

    Note: When sample volumes assayed differ from 50 µL, an appropriate mathematical adjustment must be made to accommodate the dilution factor (e.g., if 25 µL of sample is used, then calculated data must be multiplied by 2). When the sample volume assayed is less than 50 µL, compensate for the volume deficit with the standard diluent

Assay Procedure for Human MCP-1 Conferma™ ELISA Kit (Cat. # EZMCP1-99KRM)

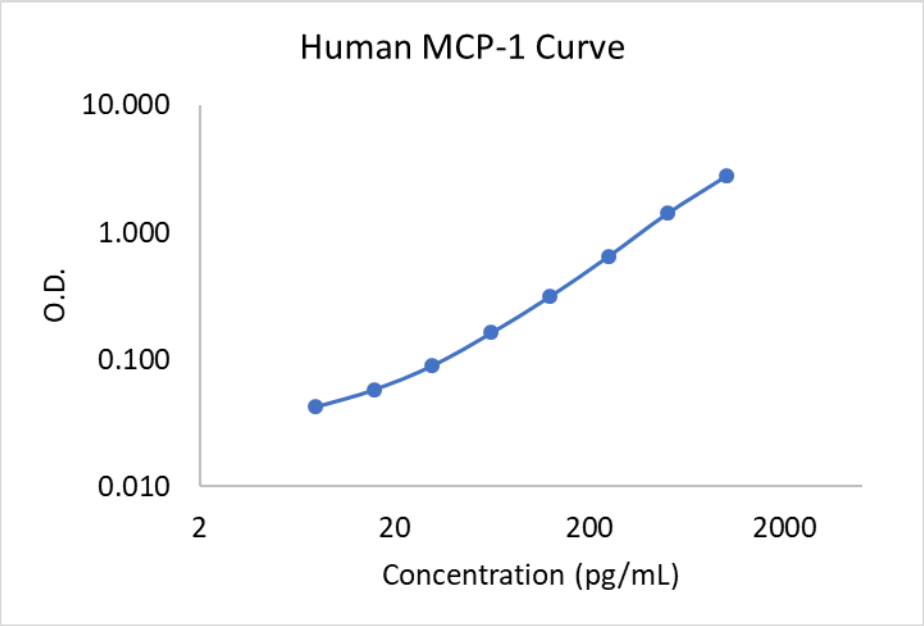
	Step 1	Step 2	Step 3	Step 4-5	Step 6-7	Step 8	Step 9	Step 10	Step 11	Step 12-13			
Well #	Wash plate 1X with 300 µL 1X Wash Buffer. Remove residual buffer by tapping smartly on absorbent towels.	Assay Buffer	Standard Diluent	Standards/ QCs/ Samples	Seal, Agitate, Incubate 2 hours at Room Temperature on a plate shaker. Wash 3X with 300 µL Wash Buffer.	Detection Antibody	Seal, Agitate, Incubate 1 hour at Room Temperature on a plate shaker. Wash 3X with 300 µL Wash Buffer.	Enzyme Solution	Seal, Agitate, Incubate 30 minutes at Room Temperature on a plate shaker. Wash 5X with 300 µL Wash Buffer.	Substrate	Seal, Agitate, Incubate for 15-25 minutes at Room Temperature on a plate shaker.	Stop	Read Absorbance at 450 nm and 590 nm.
A1, B1		50 µL	50 µL	--		100 µL		100 µL		100 µL		100 µL	
C1, D1		50 µL	--	50 µL of Std1									
E1, F1		50 µL	--	50 µL of Std 2									
G1, H1		50 µL	--	50 µL of Std 3									
A2, B2		50 µL	--	50 µL of Std 4									
C2, D2		50 µL	--	50 µL of Std 5									
E2, F2		50 µL	--	50 µL of Std 6									
G2, H2		50 µL	--	50 µL of Std 7									
A3, B3		50 µL	--	50 µL of Reconstituted standard									
C3, D3		50 µL	--	50 µL of QC 1									
E3, F3		50 µL	--	50 µL of QC 2									
G3, H3.		50 µL	--	50 µL of QC 3									
A4, B4 Etc.		50 µL	--	50 µL of diluted sample									

MICROTITER PLATE ARRANGEMENT

Human MCP-1 Conferma™ ELISA

	1	2	3	4	5	6	7	8	9	10	11	12
A	Blank	Std 4	Reconstituted Standard	Sample [#]								
B	Blank	Std 4	Reconstituted Standard	Sample [#]								
C	Std 1	Std 5	QC1									
D	Std 1	Std 5	QC1									
E	Std 2	Std 6	QC2									
F	Std 2	Std 6	QC2									
G	Std 3	Std 7	QC3									
H	Std 3	Std 7	QC3									

GRAPH OF TYPICAL REFERENCE CURVE



Typical Standard Curve, not to be used to calculate data.

ASSAY CHARACTERISTICS

A. Sensitivity

The lower limit of quantitation (LLOQ) of MCP-1 assay is 7.81 pg/mL using Belysa™ Immunoassay Analysis software from Millipore Sigma. LLOQ is calculated by back interpolation of the standard point that provides CV≤ 20% and recovery ± 20% of the expected.

B. Specificity

The antibody pair used in this assay is specific to Human MCP-1 and does not cross-react to the following molecules/hormones tested:

Human IL-1a, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-12, IL-13, IL-15, MCP-3, MIP1α, MIP1β, GROα, RANTES

C. Precision

Mean Intra-assay precision is calculated from the results of twenty replicates each of the three different concentrations of human MCP-1 in a single assay. The mean inter-assay precision is generated from the results of eight separate assays with duplicate samples in each assay for the three different concentrations of MCP-1.

ASSAY CHARACTERISTICS (continued)

Intra-Assay Variation

	Mean MCP-1 Levels (pg/mL)	Intra-Assay %CV
1	20.4	4.9
2	62.4	2.5
3	190.5	3.6

Inter-Assay Variation

	Mean MCP-1 Levels (pg/mL)	Inter-Assay %CV
1	21.4	7.6
2	62.2	3.9
3	191.1	2.3

D. Spike Recovery of MCP-1 in Blood Samples

Varying amounts of Human MCP-1 were added to 10 individual human serum and plasma samples, and the resulting MCP-1 content of each sample was assayed by Human MCP-1 Conferma™ ELISA.

The recovery = [(observed- Basal / (spiked MCP-1 concentration)] x 100%.

Sample	Spiked Concentration of MCP-1 (pg/mL)	Concentration observed in assay (pg/mL)	Recovery%
Serum 1	0	36.8	
	31.25	63.2	85
	62.5	90.6	86
	125	147.6	89
Serum 2	0	31.3	
	31.25	56.6	81
	62.5	79.9	78
	125	133.8	82
Serum 3	0	18.5	
	31.25	45.2	85
	62.5	71.3	85
	125	128.4	88

Sample	Spiked Concentration of MCP-1 (pg/mL)	Concentration observed in assay (pg/mL)	Recovery%
Serum 4	0	27.2	
	31.25	54.4	87
	62.5	80.8	86
	125	138.8	89
Serum 5	0	42.3	
	31.25	68.9	85
	62.5	101.4	95
	125	158.8	93
Average			86

Sample	Spiked Concentration of MCP-1 (pg/mL)	Concentration observed in assay (pg/mL)	Recovery%
Plasma 1	0	23.0	
	31.25	51.0	90
	62.5	69.9	75
	125	128.2	84
Plasma 2	0	18.9	
	31.25	45.9	87
	62.5	78.0	95
	125	125.9	86
Plasma 3	0	25.3	
	31.25	54.6	94
	62.5	79.4	87
	125	137.8	90
Plasma 4	0	21.6	
	31.25	50.3	92
	62.5	75.7	87
	125	131.6	88
Plasma 5	0	15.9	
	31.25	42.8	86
	62.5	69.6	86
	125	124.5	87
Average			87

ASSAY CHARACTERISTICS (continued)

E. Linearity of Dilution

10 spiked individual human serum and plasma samples were assayed for linearity studies. Neat sample volumes of 10 µL, 5 µL, 2.5 µL, and 1.25 µL in a 50 µL total sample volume represents dilution factors of 1, 2, 4, and 8, respectively. Required amounts of Standard Diluent were added to compensate for the lost volumes below 10 µL.

Dilution linearity= (observed/expected) x 100%
Observed= mean calculated dilution corrected concentration at each dilution
Expected= mean calculated concentration of the sample at the recommended dilution

Sample	Neat Sample volume in 50 µl total volume (µl)	Mean (pg/mL)	Dilution Corrected (pg/mL)	Linearity%
Serum 1	10	147.7	147.7	
	5	69.9	139.9	95
	2.5	35.0	140.1	95
	1.25	18.7	149.7	101
Serum 2	10	145.5	145.5	
	5	68.9	137.7	95
	2.5	35.0	140.1	96
	1.25	19.0	151.7	104
Serum 3	10	128.0	128.0	
	5	61.2	122.5	96
	2.5	31.3	125.3	98
	1.25	17.0	136.0	106
Serum 4	10	147.3	147.3	
	5	72.1	144.2	98
	2.5	36.9	147.5	100
	1.25	19.0	151.7	103
Serum 5	10	135.2	135.2	
	5	65.0	129.9	96
	2.5	32.4	129.5	96
	1.25	15.8	126.8	94
Average				98

ASSAY CHARACTERISTICS (continued)

Sample	Neat Sample volume in 50 µl total volume (µl)	Mean (pg/mL)	Dilution Corrected (pg/mL)	Linearity%
Plasma 1	10	131.1	131.1	
	5	63.2	126.4	96
	2.5	31.8	127.2	97
	1.25	17.0	136.0	104
Plasma 2	10	133.7	133.7	
	5	66.9	133.8	100
	2.5	33.6	134.6	101
	1.25	17.7	141.9	106
Plasma 3	10	124.9	124.9	
	5	60.8	121.6	97
	2.5	30.6	122.5	98
	1.25	17.2	137.9	110
Plasma 4	10	139.8	139.8	
	5	66.9	133.8	96
	2.5	32.7	130.9	94
	1.25	17.7	141.9	101
Plasma 5	10	137.8	137.8	
	5	67.6	135.1	98
	2.5	34.3	137.4	100
	1.25	18.7	149.7	109
Average				100

NOTE: More data related to assay characteristics can be found in Human MCP-1 Conferma™ ELISA verification report.

QUALITY CONTROLS

The ranges for Quality Control 1, 2, and 3 are provided on the card insert or can be located at the MILLIPORE SIGMA website www.milliporesigma.com.

TROUBLESHOOTING GUIDE

1. To obtain reliable and reproducible results, the operator should carefully read this manual and fully understand all aspects of each assay step before attempting to run the assay.
2. Throughout the assay, the operator should adhere strictly to the procedures with good laboratory practice.
3. Have all necessary reagents and equipment ready on hand before starting. Once the assay has been started, all steps should be completed with precise timing and without interruption.
4. Avoid cross-contamination of any reagents or samples to be used in the assay.
5. Make sure all reagents and samples are added to the bottom of each well.
6. Careful and complete mixing of solutions in the well is critical. Poor assay precision will result from incomplete mixing or cross well contamination due to inappropriate mixing.
7. Remove any air bubbles formed in the well after acidification of the substrate solution because bubbles interfere with spectrophotometric readings.
8. High signal in the background or blank wells could be due to 1.) cross well contamination by standard solution or sample or 2.) inadequate washing of wells with Wash Buffer or 3.) overexposure to light after the substrate has been added.

ORDERING INFORMATION

To place an order or to obtain additional information about our immunoassay products, please contact your Customer Service or Technical Support Specialist. Contact information for each region can be found on our website:

emdmillipore.com/contact

Conditions of Sale

For Research Use Only. Not for Use in Diagnostic Procedures.

Safety Data Sheets (SDS)

Safety Data Sheets for EMD Millipore products may be ordered by fax or phone or through our website at emdmillipore.com/msds.