

# Quantikine<sup>®</sup> ELISA

## Human CCL2/MCP-1 Immunoassay

Catalog Number DCP00

SCP00

PDCP00

For the quantitative determination of human Monocyte Chemotactic Protein 1 (MCP-1) concentrations in cell culture supernates, serum, plasma, and urine.

This package insert must be read in its entirety before using this product.  
For research use only. Not for use in diagnostic procedures.

# TABLE OF CONTENTS

SECTION	PAGE
INTRODUCTION .....	1
PRINCIPLE OF THE ASSAY.....	2
LIMITATIONS OF THE PROCEDURE .....	2
TECHNICAL HINTS.....	2
MATERIALS PROVIDED & STORAGE CONDITIONS .....	3
OTHER SUPPLIES REQUIRED .....	4
PRECAUTIONS.....	4
SAMPLE COLLECTION & STORAGE.....	4
SAMPLE PREPARATION.....	5
REAGENT PREPARATION .....	5
ASSAY PROCEDURE .....	6
CALCULATION OF RESULTS.....	7
TYPICAL DATA.....	7
PRECISION .....	8
RECOVERY.....	8
SENSITIVITY .....	8
LINEARITY.....	9
CALIBRATION .....	9
SAMPLE VALUES.....	10
SPECIFICITY.....	10
REFERENCES .....	11
PLATE LAYOUT .....	13

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## INTRODUCTION

Monocyte Chemoattractant Protein-1 (MCP-1), also known as CCL2, MCAF and TDCF, is a heparin-binding, 10-14 kDa member of the beta or CC family of chemokines. Currently, there are at least 26 human CC family members that generally range from 8-12 kDa in size. The majority of these chemokines are found on human chromosome 17 and all contain a typical three  $\beta$ -sheet/one  $\alpha$ -helix structure (1-3). Human MCP-1 is synthesized as a 99 amino acid (aa) precursor that contains a 23 aa signal sequence coupled to a 76 aa mature region (4-6). The mature region contains a receptor binding and dimerization N-terminus plus a glycosaminoglycan (GAG)-binding C-terminus (2, 7). MCP-1 undergoes O-linked but not N-linked glycosylation, and variability in the pattern of glycosylation accounts for variability in its MW (4, 5, 8, 9). Heavily glycosylated forms of MCP-1 show increased half-life, while lightly glycosylated forms of MCP-1 show increased bioactivity (10). MCP-1 circulates as a monomer and is also suggested to form dimers and/or multimers (11-13). The monomer is considered the predominant form and demonstrates full chemotactic activity (11, 12). Dimers and higher order forms are suggested to serve as a link between circulating cells and the surface of vascular endothelium (13). The mouse ortholog to human MCP-1 is termed JE, which contains a 49 aa extension at the C-terminus. Over their shared aa sequences, mature human MCP-1 and mouse JE share 57% aa sequence identity (14). Mature human and porcine MCP-1 aa sequences are 79% identical (15). Multiple isoforms of MCP-1 exist that are generated through proteolytic processing. Although MCP-1 is not subject to CD26/DPPIV N-terminal processing, it does undergo MMP processing by MMP-1, -2, -3, and -9 (8, 16, 17). Truncation at the N-terminus creates a 72 aa isoform (aa 28-99) that retains some bioactivity, a 71 aa isoform (aa 29-99) that shows no activity, and a C-terminally processed isoform (aa 24-92) that possesses full bioactivity (9, 16, 17). A wide variety of cells secrete MCP-1, including endothelial cells (EC), monocytes, fibroblasts, and vascular smooth muscle cells (1, 4, 5), mast cells (18), and astrocytes (19).

There are three G-protein-coupled receptors for MCP-1: CCR2, CCR4 and D6/CCBP2 (1, 20-22). D6 is considered a decoy receptor and likely does not signal. CCR2 has two isoforms (A and B) that differ in their cytoplasmic tails and are differentially expressed. The A isoform is found on T cells and smooth muscle cells and does not mobilize calcium, while the B isoform is found on monocytes and activated NK cells and does mobilize calcium. CCR4 is expressed on a wide variety of hematopoietic cells. MCP-1 is best known for its chemotactic activity on monocytes. When secreted by EC, it likely binds to heparin sulfate on the EC where it oligomerizes and forms an attachment point for CCR2 on circulating monocytes (7, 13). This promotes tethering and subsequent migration. When secreted by cells at sites of inflammation, it induces their chemotaxis into the area. This migration is accompanied by an initial release of leukocyte-derived MMPs that create a pathway through the ECM. These MMPs do not act on MCP-1. However, upon arrival at their destination, new MMPs (such as MMP-1 and -3) are released by leukocytes that do act on MCP-1, inactivating it and providing a brake on the inflammatory process (16).

Several different lines of evidence utilizing *in vivo* animal models suggest that MCP-1 is an important player in inflammatory processes. Blocking MCP-1/JE activity can suppress models of endotoxemia, delayed-type sensitivity reactions, and inflammatory arthritis, while over-expression enhances the recruitment of monocytes and lymphocytes (23-26). In contrast, several knockout studies show that MCP-1/JE-deficient mice exhibit suppressed inflammation-related macrophage, monocyte, NK cell, NKT cell, and/or  $\gamma\delta$  T cell infiltration in several different contexts including models of pulmonary infection, stroke, blood vessel injury, renal tubule injury, autoimmune disease, uveitis, and wound healing (27-36). Other putative functions revealed by MCP-1/JE-deficient mice include roles in angiogenesis and the Th2 polarization of naive T cells (36, 37). Elevated MCP-1 levels in humans have been associated with sepsis, Crohn's disease, lupus nephritis, amyotrophic lateral sclerosis, multiple sclerosis, rheumatoid arthritis, acute pancreatitis, and atherosclerosis (38-44). MCP-1 is also upregulated in several cancers including gastric carcinoma, esophageal squamous cell carcinoma, malignant glioma, and ovarian, pancreatic, bladder, and breast cancers (45-50).

The Quantikine® Human MCP-1 Immunoassay is a 3.5-4.5 hour solid phase ELISA designed to measure human MCP-1 in cell culture supernates, serum, plasma, and urine. It contains *E. coli*-expressed recombinant human MCP-1 and antibodies raised against the recombinant factor. This immunoassay has been shown to accurately quantitate recombinant human MCP-1. Results obtained using natural human MCP-1 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 MCP-1.

## PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for human MCP-1 has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any MCP-1 present is bound by the immobilized antibody. After washing away any unbound substances, an enzyme-linked polyclonal antibody specific for human MCP-1 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 MCP-1 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.
- It is important that the calibrator diluent selected for the standard curve be consistent with the samples being assayed.
- If samples generate values higher than the highest standard, further dilute the samples with the appropriate calibrator diluent and repeat the assay. If cell culture supernate samples require a large dilution, perform an intermediate dilution with culture media and the final dilution with Calibrator Diluent RD5L (diluted 1:5).
- 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 #	CATALOG # DCP00	CATALOG # SCP00	DESCRIPTION	STORAGE OF OPENED/ RECONSTITUTED MATERIAL
Human MCP-1 Microplate	890223	1 plate	6 plates	96 well polystyrene microplate (12 strips of 8 wells) coated with a monoclonal antibody specific for human MCP-1.	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 MCP-1 Standard	890225	1 vial	6 vials	Recombinant human MCP-1 in a buffered protein base with preservatives; lyophilized. <i>Refer to the vial label for reconstitution volume.</i>	Aliquot and store for up to 1 month at ≤ -20 °C* in a manual defrost freezer. Avoid repeated freeze-thaw cycles.
Human MCP-1 Conjugate	890224	1 vial	6 vials	21 mL/vial of a polyclonal antibody specific for human MCP-1 conjugated to horseradish peroxidase with preservatives.	May be stored for up to 1 month at 2-8 °C.*
Assay Diluent RD1-83	895875	1 vial	6 vials	11 mL/vial of a buffered protein base with preservatives. <i>For serum/plasma samples. May contain a precipitate. Mix well before and during use.</i>	
Calibrator Diluent RD5L	895028	1 vial	6 vials	21 mL/vial of a concentrated buffered protein base with preservatives. <i>For cell culture supernate/urine samples. Use diluted 1:5 in this assay.</i>	
Calibrator Diluent RD6Q	895128	2 vials	12 vials	21 mL/vial of animal serum with preservatives. <i>For serum/plasma samples.</i>	
Wash Buffer Concentrate	895003	1 vial	6 vials	21 mL/vial of a 25-fold concentrated solution of buffered surfactant with preservative. <i>May turn yellow over time.</i>	
Color Reagent A	895000	1 vial	6 vials	12 mL/vial of stabilized hydrogen peroxide.	
Color Reagent B	895001	1 vial	6 vials	12 mL/vial of stabilized chromogen (tetramethylbenzidine).	
Stop Solution	895032	1 vial	6 vials	6 mL/vial of 2 N sulfuric acid.	
Plate Sealers	N/A	4 strips	24 strips	Adhesive strips.	

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

DCP00 contains sufficient materials to run an ELISA on one 96 well plate.

SCP00 (SixPak) contains sufficient materials to run ELISAs on six 96 well plates.

This kit is also available in a PharmPak (R&D Systems®, Catalog # PDCP00). PharmPaks contain sufficient materials to run ELISAs on 50 microplates. Specific vial counts of each component may vary. Refer to the literature accompanying your order for specific vial counts.

## 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.
- 100 mL and 500 mL graduated cylinders.
- Deionized or distilled water.
- Squirt bottle, manifold dispenser, or automated microplate washer.
- **Polypropylene** test tubes for dilution of standards and samples.
- Human MCP-1 controls (optional; R&D Systems®, Catalog # QC01-1).

## PRECAUTIONS

Some components of this kit contain sodium azide which may react with lead and copper plumbing to form explosive metallic azides. Flush with large volumes of water during disposal.

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 MSDS 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** - To prevent loss of MCP-1 in culture supernates prior to assay, it is recommended that media be supplemented with at least 2% fetal bovine serum. Remove particulates by centrifugation and assay immediately or aliquot and store samples at  $\leq -20$  °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  $\leq -20$  °C. Avoid repeated freeze-thaw cycles.

**Plasma** - Collect plasma using EDTA, heparin, or citrate as an anticoagulant. Centrifuge for 15 minutes at 1000 x g within 30 minutes of collection. Assay immediately or aliquot and store samples at  $\leq -20$  °C. Avoid repeated freeze-thaw cycles.

**Urine** - Aseptically collect the first urine of the day (mid-stream), voided directly into a sterile container. Centrifuge to remove particulate matter. Assay immediately or aliquot and store at  $\leq -20$  °C. Avoid repeated freeze-thaw cycles.

## SAMPLE PREPARATION

**Warning: Polypropylene tubes must be used. Do not use glass.**

All serum and plasma samples require at least a 2-fold dilution into Calibrator Diluent RD6Q. A suggested dilution is 250  $\mu$ L of sample + 250  $\mu$ L of Calibrator Diluent RD6Q.

All urine samples require at least a 2-fold dilution into Calibrator Diluent RD5L (diluted 1:5). A suggested dilution is 250  $\mu$ L of sample + 250  $\mu$ L of Calibrator Diluent RD5L (diluted 1:5).

## REAGENT PREPARATION

**Bring all reagents to room temperature before use.**

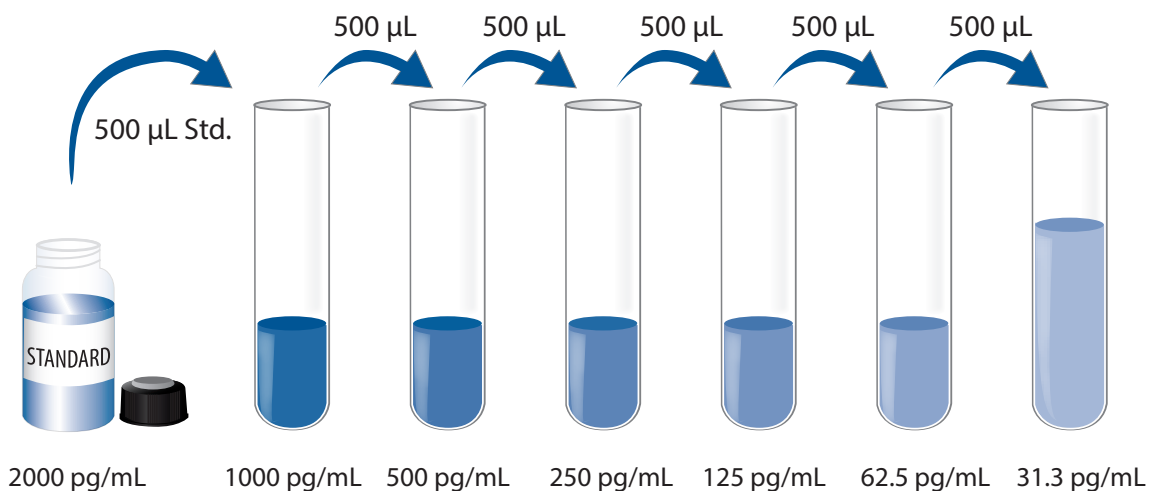
**Wash Buffer** - If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. Add 20 mL of Wash Buffer Concentrate to deionized or distilled water to prepare 500 mL of Wash Buffer.

**Calibrator Diluent RD5L (diluted 1:5)** - Add 20 mL of Calibrator Diluent RD5L to deionized or distilled water to prepare 100 mL of Calibrator Diluent RD5L (diluted 1:5).

**Substrate Solution** - Color Reagents A and B should be mixed together in equal volumes within 15 minutes of use. Protect from light. 200  $\mu$ L of the resultant mixture is required per well.

**Human MCP-1 Standard - Refer to the vial label for reconstitution volume.** Reconstitute the Human MCP-1 Standard with Calibrator Diluent RD5L (diluted 1:5) (*for cell culture supernate/urine samples*) or Calibrator Diluent RD6Q (*for serum/plasma samples*). This reconstitution produces a stock solution of 2000 pg/mL. Allow the standard to sit for a minimum of 15 minutes with gentle agitation prior to making dilutions.

**Use polypropylene tubes.** Pipette 500  $\mu$ L of Calibrator Diluent RD5L (diluted 1:5) (*for cell culture supernate/urine samples*) or Calibrator Diluent RD6Q (*for serum/plasma samples*) into each tube. Use the stock solution to produce a dilution series (below). Mix each tube thoroughly before the next transfer. The undiluted Human MCP-1 Standard (2000 pg/mL) serves as the high standard. The appropriate calibrator diluent 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, samples, and controls be assayed in duplicate.**

1. Prepare all reagents, working standards, 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. **For Serum/Plasma Samples Only:** Add 50  $\mu\text{L}$  of Assay Diluent RD1-83 to each well. *Assay Diluent RD1-83 may contain a precipitate. Mix well before and during use.*
4. Add 200  $\mu\text{L}$  of standard, sample\*, or control per well. Cover with the adhesive strip provided. Incubate for 2 hours at room temperature. A plate layout is provided to record standards and samples assayed.
5. Aspirate each well and wash, repeating the process twice for a total of three washes. Wash by filling each well with Wash Buffer (400  $\mu\text{L}$ ) 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  $\mu\text{L}$  of Human MCP-1 Conjugate to each well. Cover with a new adhesive strip.  
**For Cell Culture Supernate/Urine Samples:** Incubate for 1 hour at room temperature.  
**For Serum/Plasma Samples:** Incubate for 2 hours at room temperature.
7. Repeat the aspiration/wash as in step 5.
8. Add 200  $\mu\text{L}$  of Substrate Solution to each well. Incubate for 30 minutes at room temperature. **Protect from light.**
9. Add 50  $\mu\text{L}$  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.

\*Samples may require a 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.).

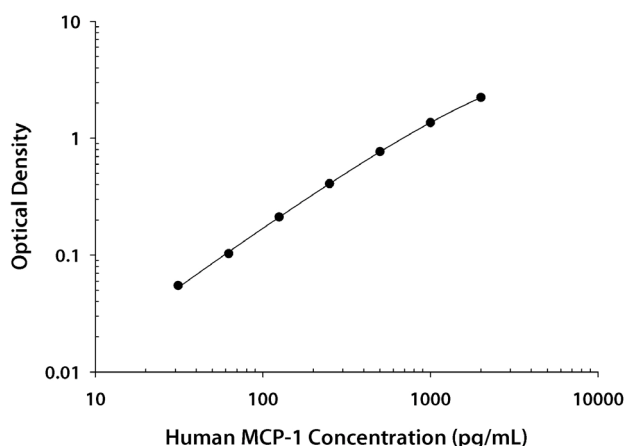
Create a standard curve by reducing the data using computer software capable of generating a four parameter logistic (4-PL) curve-fit. As an alternative, construct a standard curve by plotting the mean absorbance for each standard on the y-axis against the concentration on the x-axis and draw a best fit curve through the points on the graph. The data may be linearized by plotting the log of the human MCP-1 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 prior to the assay, the measured concentrations must be multiplied by the dilution factor.

## TYPICAL DATA

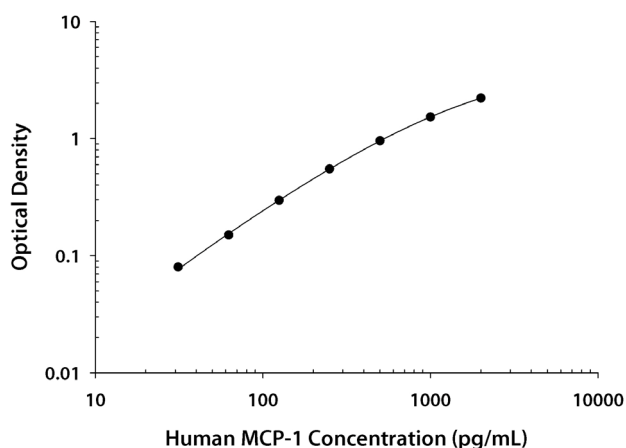
These standard curves are provided for demonstration only. A standard curve should be generated for each set of samples assayed.

### CELL CULTURE SUPERNATE/URINE ASSAY



(pg/mL)	O.D.	Average	Corrected
0	0.052 0.055	0.054	—
31.3	0.108 0.110	0.109	0.055
62.5	0.155 0.159	0.157	0.103
125	0.260 0.273	0.266	0.212
250	0.449 0.476	0.462	0.408
500	0.806 0.834	0.820	0.766
1000	1.397 1.429	1.413	1.359
2000	2.247 2.317	2.282	2.228

### SERUM/PLASMA ASSAY



(pg/mL)	O.D.	Average	Corrected
0	0.029 0.031	0.030	—
31.3	0.109 0.112	0.110	0.080
62.5	0.178 0.181	0.180	0.150
125	0.323 0.331	0.327	0.297
250	0.578 0.582	0.580	0.550
500	0.959 1.015	0.987	0.957
1000	1.518 1.597	1.558	1.528
2000	2.221 2.261	2.241	2.211

## 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.

## CELL CULTURE SUPERNATE/URINE ASSAY

Sample	Intra-Assay Precision			Inter-Assay Precision		
	1	2	3	1	2	3
n	20	20	20	20	20	20
Mean (pg/mL)	78.2	360	1079	76.0	360	1086
Standard deviation	3.3	17.7	63.5	4.5	17.2	48.5
CV (%)	4.2	4.9	5.9	5.9	4.8	4.5

## SERUM/PLASMA ASSAY

Sample	Intra-Assay Precision			Inter-Assay Precision		
	1	2	3	1	2	3
n	20	20	20	20	20	20
Mean (pg/mL)	76.7	364	1121	74.2	352	1076
Standard deviation	6.0	17.1	54.4	5.0	20.5	49.4
CV (%)	7.8	4.7	4.9	6.7	5.8	4.6

## RECOVERY

The recovery of human MCP-1 spiked to three levels throughout the range of the assay was evaluated.

Sample Type	Average % Recovery	Range
Cell culture media (n=5)	96	88-107%
Serum* (n=5)	103	92-113%
EDTA plasma* (n=5)	96	92-102%
Heparin plasma* (n=5)	102	94-114%
Citrate plasma* (n=5)	100	94-107%
Urine* (n=5)	92	85-100%

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

## SENSITIVITY

Thirty-four assays were evaluated and the minimum detectable dose (MDD) of human MCP-1 ranged from 0.57-10.0 pg/mL. The mean MDD was 1.7 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.

## LINEARITY

To assess the linearity of the assay, samples were spiked with high concentrations of human MCP-1 and diluted with the appropriate calibrator diluent to produce samples with values within the dynamic range of the assay.

		Cell culture media (n=5)	Serum* (n=5)	EDTA plasma* (n=5)	Heparin plasma* (n=5)	Citrate plasma* (n=5)	Urine* (n=5)
1:2	Average % of Expected	102	99	96	98	95	95
	Range (%)	98-106	97-101	92-99	89-103	91-97	89-108
1:4	Average % of Expected	102	97	96	96	95	92
	Range (%)	97-108	91-103	90-106	88-102	91-100	87-102
1:8	Average % of Expected	103	97	98	96	96	91
	Range (%)	96-109	90-102	92-108	89-103	89-106	88-97
1:16	Average % of Expected	101	95	94	98	94	90
	Range (%)	93-109	84-106	87-98	87-107	89-100	83-99

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

## CALIBRATION

This immunoassay is calibrated against a highly purified *E. coli*-expressed recombinant human MCP-1 produced at R&D Systems®. The NIBSC MCP-1 non-WHO Reference Standard 92/794 (Human rDNA derived) was evaluated in this kit.

The dose response curve of the reference standard 92/794 parallels the Quantikine® standard curve. To convert sample values obtained with the Quantikine® Human MCP-1 kit to approximate NIBSC 92/794 units use the equation below.

NIBSC (92/794) approximate value (U/mL) = 0.00085 x Quantikine® Human MCP-1 value (pg/mL)

## SAMPLE VALUES

**Serum/Plasma/Urine** - Samples from apparently healthy volunteers were evaluated for the presence of human MCP-1 in this assay. No medical histories were available for the donors used in this study. The reported urine values are actual and are not normalized for creatinine content.

Sample Type	Mean (pg/mL)	Range (pg/mL)
Serum (n=37)	370	200-722
EDTA plasma (n=37)	153	72-295
Citrate plasma (n=37)	196	134-436
Heparin plasma (n=37)	242	113-340
Urine (n=37)	211	42-410

**Cell Culture Supernates** - Human peripheral blood leukocytes were cultured in RPMI 1640 and supplemented with 10% fetal bovine serum. The cells were cultured unstimulated or stimulated with 10 µg/mL PHA for 2 or 5 days. Aliquots of the cell culture supernates were removed and assayed for levels of human MCP-1.

Condition	Day 2 (pg/mL)	Day 5 (pg/mL)
Unstimulated	647	1785
Stimulated	67,225	70,000

## SPECIFICITY

This assay recognizes natural and recombinant human MCP-1.

The following factors were prepared at 1.0 ng/mL, 10 ng/mL, and 50 ng/mL in calibrator diluent and assayed for cross-reactivity. Individual preparations of the same factors at 1.0 ng/mL, 10 ng/mL, and 50 ng/mL in a mid range recombinant human MCP-1 control were also assayed for interference. No significant cross-reactivity or interference was observed.

### Recombinant human:

GRO $\alpha$   
GRO $\beta$   
GRO $\gamma$   
IL-8  
MCP-2  
MCP-3  
MIP-1 $\alpha$   
MIP-1 $\beta$   
RANTES

### Recombinant mouse:

JE/MCP-1  
MIP-1 $\alpha$   
MIP-1 $\beta$

### Recombinant rat:

JE/MCP-1

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**PLATE LAYOUT**

Use this plate layout to record standards and samples assayed.

12									
11									
10									
9									
8									
7									
6									
5									
4									
3									
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	A	B	C	D	E	F	G	H	



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