PRODUCT INFORMATION & MANUAL

Human CCL2/MCP-1 Valukine™ ELISA

Catalog Number: VAL206

For the quantitative determination of natural and recombinant human Monocyte Chemotactic Protein 1 (MCP-1)/CCL2 concentrations

For research use only.
Not for diagnostic or therapeutic procedures.

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Please refer to the kit label for expiry date.
Novus kits are guaranteed for 3 months from date of receipt

Version 202411.1

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I. BACKGROUND

Monocyte Chemotactic Protein-1 (MCP-1), also known as CCL2, MCAF and TDCF, is a heparinbinding, 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 β-sheet/one α-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 γδ 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).

II. OVERVIEW

A. PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. An antibody specific for human MCP-1 has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any human MCP-1 present is bound by the immobilized antibody. After washing away any unbound substances, an enzyme-linked antibody specific for human MCP-1 is added to the wells. Following a wash to remove any unbound antibody-enzyme reagent, TMB substrate (Chromogenic agent) is added to the wells and color develops in proportion to the amount of human MCP-1 bound in the initial step. The color development is stopped and the intensity of the color is measured.

B. LIMITATIONS OF THE PROCEDURE

- **FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.**
- This kit is suitable for cell culture supernate, human serum, human plasma and human urine.
- 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 (1×)/Calibrator Diluent RD6Q and repeat the assay.
- Any variation in operator, pipetting technique, washing technique, incubation time or temperature, and kit age can cause variation in binding.

III. ADVANTAGES

A. PRECISION

Intra-assay Precision (Precision within an assay)

Three samples were tested twenty times on one plate to assess intra-assay precision.

Inter-assay Precision (Precision between assays)

Three samples were tested in twenty separate assays to assess inter-assay precision.

CELL CULTURE SUPERNATE/URINE ASSAY

	Intra-assay Precision			**Inter-assay Precision**		
Sample	1	2	3	1	2	3
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

	Intra-assay Precision			**Inter-assay Precision**		
Sample	1	2	3	1	2	3
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

B. RECOVERY

The recovery of Human MCP-1 spiked to three levels throughout the range of the assay in various matrices was evaluated.

Sample Type	Average % Recovery	Range (%)
Cell culture media (n=5)	96	88-107
Human serum* (n=5)	103	92-113
Human EDTA plasma* (n=5)	96	92-102
Human heparin plasma* (n=5)	102	94-114
Human citrate plasma* (n=5)	100	94-107
Human urine* (n = 5)	92	85-100

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

C. 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 optical density value of twenty zero standard replicates and calculating the corresponding concentration.

D. 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 Valukine standard curve. To convert sample values obtained with the Valukine Human MCP-1 kit to approximate NIBSC 92/794 units use the equation below.

NIBSC (92/794) approximate value (U/mL) = 0.002 x Valukine Human MCP-1 value (pg/mL)

Note: *Based on data generated in May 2019.*

E. LINEARITY

To assess the linearity of the assay, different samples were containing or spiked with high concentrations of human MCP-1 and diluted with Calibrator Diluent (1×)/Calibrator Diluent RD6Q to produce samples with values within the dynamic range of the assay.

Dilution		Cell culture media (n=5)	Human serum* (n=5)	Human EDTA plasma* (n=5)	Human heparin plasma* (n=5)	Human citrate plasma* (n=5)	Human 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.

F. SAMPLE VALUES

Human 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)
Human serum (n=37)	370	200-722
Human EDTA plasma (n=37)	153	72-295
Human citrate plasma (n=37)	196	134-436
Human heparin plasma (n=37)	242	113-340
Human 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	67225	70000

G. 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 (1×)/Calibrator Diluent RD6Q 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.

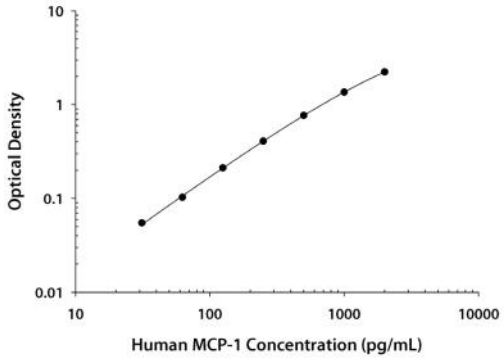
Recombinant human：		**Recombinant mouse：**
GROα	MCP-3	JE/MCP-1
GROβ	MIP-1α	MIP-1α
GROγ	MIP-1β	MIP-1β
IL-8	RANTES	**Recombinant rat：**
MCP-2		JE/MCP-1

IV. EXPERIMENT

EXAMPLE STANDARD

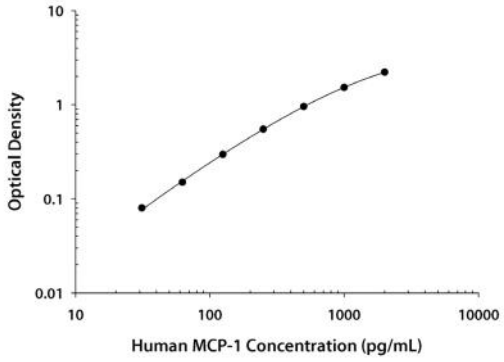
These standard curves are provided for demonstration only. A standard curve should be generated for each set of samples assayed.

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

V. KIT COMPONENTS AND STORAGE

A. MATERIALS PROVIDED

Parts	Description	Size
Human MCP-1 Microplate	96 well polystyrene microplate (12 strips of 8 wells) coated with an antibody against human MCP-1	1 plate
Human MCP-1 Conjugate	An antibody specific for human MCP-1 conjugated to horseradish peroxidase	1 vial
Human MCP-1 Standard	Recombinant human MCP-1 in a buffered protein base; lyophilized. Refer to the vial label for reconstitution volume	1 vial
Assay Diluent RD1-83	A buffered protein base	1 vial
Calibrator Diluent Concentrate (5×)/ RD5L	A 5× concentrated buffered protein base used to dilute standard and samples (For cell culture supernate/urine samples)	1 vial
Calibrator Diluent RD6Q	Animal serum used to dilute standard and samples (For serum/plasma samples)	2 vials
Wash Buffer Concentrate (25×)	A 25× concentrated solution of buffered surfactant	1 vial
TMB Substrate	TMB ELISA Substrate Solution/TMB Substrate Solution	2 vials
Stop Solution	2 N sulfuric acid	1 vial
Plate Sealers	Adhesive strip	3 strips

B. STORAGE

<table>
<tr><td>Unopened Kit</td><td colspan="2">Store at 2-8°C. Do not use past kit expiration date.</td></tr>
<tr><td rowspan="8">Opened/ Reconstituted Reagents</td><td>Wash Buffer (1×)</td><td rowspan="5">May be stored for up to 1 month at 2-8°C.*</td></tr>
<tr><td>Stop Solution</td></tr>
<tr><td>Assay Diluent RD1-83</td></tr>
<tr><td>Calibrator Diluent RD6Q</td></tr>
<tr><td>Conjugate</td></tr>
<tr><td>TMB Substrate</td><td rowspan="2">Aliquot and store for up to 1 month at ≤ -20 °C* in a manual defrost freezer. Avoid repeated freeze-thaw cycles.</td></tr>
<tr><td>Standard</td></tr>
<tr><td>Calibrator Diluent Concentrate (5×)/ RD5L</td><td>May be stored for up to 1 month at 2-8 °C.*
Use and discard diluted Calibrator Diluent (1×). Prepare fresh for each assay.</td></tr>
<tr><td>Microplate Wells</td><td>Return unused wells to the foil pouch containing the desiccant pack, reseal along entire edge of zip-seal. May be stored for up to 1 month at 2-8°C.*</td></tr>
</table>

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

C. 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.
- 100 mL and 500 mL graduated cylinder.
- Polypropylene test tubes for dilution of standards and samples.

D. PRECAUTION

- Some components in this kit contain a preservative which may cause an allergic skin reaction. Avoid breathing mist.
- 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. Wear eye, hand, face, and clothing protection when using this material.

VI. PREPARATION

A. SAMPLE COLLECTION AND 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 ≤ -20 °C. Avoid repeated freeze-thaw cycles. Samples may require dilution with Calibrator Diluent (1×).

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 × g. Remove serum and assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles. Samples may require dilution with Calibrator Diluent RD6Q.

Plasma - Collect plasma using EDTA, heparin or citrate as an anticoagulant. Centrifuge for 15 minutes at 1000 × g within 30 minutes of collection. Assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles. Samples may require dilution with Calibrator Diluent RD6Q.

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 ≤ -20 °C. Avoid repeated freeze-thaw cycles. Samples may require dilution with Calibrator Diluent (1×).

B. SAMPLE PREPARATION

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

All human serum and plasma samples recommend a 2-fold dilution. A suggested 2-fold dilution is 250 µL of sample + 250 µL of Calibrator Diluent RD6Q. Optimal dilutions should be determined by the end user.

All human urine samples recommend a 2-fold dilution. A suggested 2-fold dilution is 250 µL of sample + 250 µL of Calibrator Diluent (1×). Optimal dilutions should be determined by the end user.

C. REAGENT PREPARATION

Bring all reagents to room temperature before use.

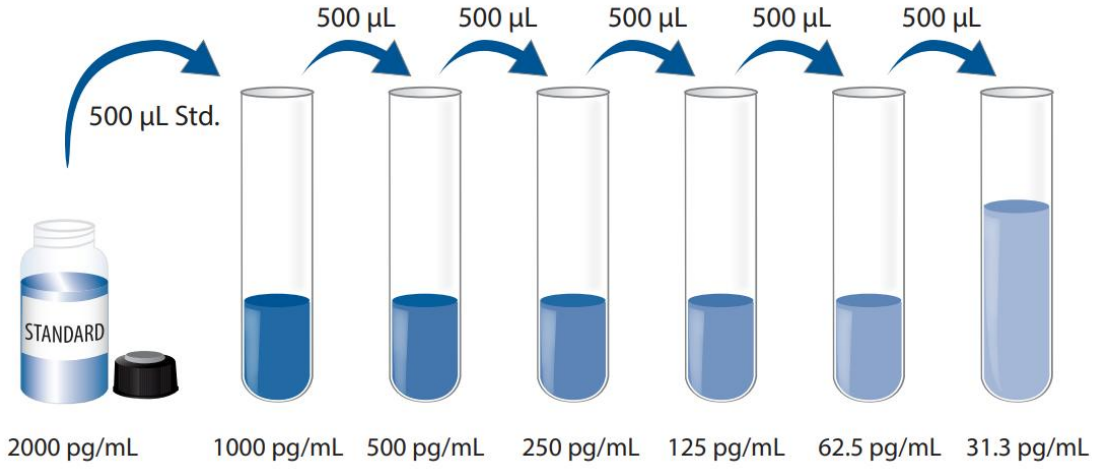
Wash Buffer (1×) - If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. Dilute 20 mL of Wash Buffer Concentrate (25×) into deionized or distilled water to prepare 500 mL of Wash Buffer (1×).

Calibrator Diluent (1×) - Use deionized or distilled water to prepare Calibrator Diluent (1×).

Human MCP-1 Standard - Refer to the vial label for the reconstitution volume* Reconstitute the human MCP-1 Standard with Calibrator Diluent (1×) (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.

*If you have any question, please seek help from our Technical Support.

Use polypropylene tubes. Pipette 500 µL of Calibrator Diluent (1×) (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 (1×) (for cell culture supernate/urine samples) or Calibrator Diluent RD6Q (for serum/plasma samples) serves as the zero standard (0 pg/mL).



D. 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.
- It is recommended that the samples be pipetted within 15 minutes.
- To ensure accurate results, proper adhesion of plate sealers during incubation steps is necessary.
- TMB Substrate should remain colorless until added to the plate. Keep TMB Substrate protected from light. TMB Substrate should change from colorless to gradations of blue.
- Stop Solution should be added to the plate in the same order as the TMB Substrate. 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 TMB Substrate.

VII.ASSAY PROCEDURE

Bring all other reagents and samples to room temperature before use. It is recommended that all standards and samples 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 µL 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 µL of standard and prepared sample per well. Cover with the adhesive strip provided. **Incubate for 2 hours at room temperature.** A plate layout is provided for a record of standards and samples assayed.
5. Aspirate each well and wash, repeating the process two times for a total of three 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 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 µL of TMB Substrate to each well. **Incubate for 30 minutes at room temperature. Protect from light**.
9. Add 50 µL of Stop Solution to each well. Gently tap the plate to ensure thorough mixing.
10. Determine the optical density of each well within 10 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.

11. CALCULATION OF RESULTS

Average the duplicate readings for each standard 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, the concentration read from the standard curve must be multiplied by the dilution factor.

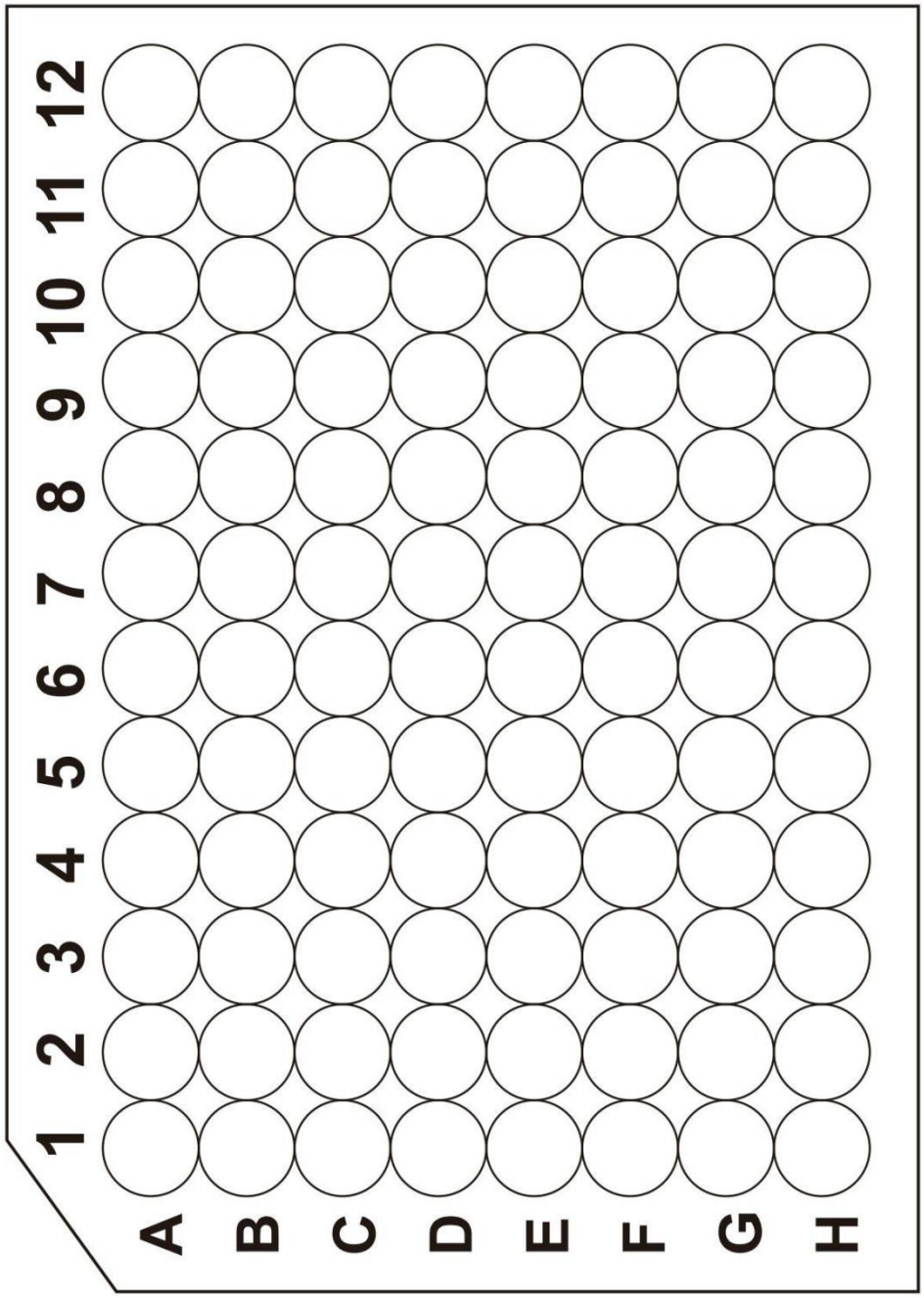
VIII. REFERENCES

1. Deshmane, S.L. et al. (2009) J. Interferon Cytokine Res. 29:313.
2. van Coillie, E. et al. (1999) Cytokine Growth Factor Res. 10:61.
3. Sharma, M. et al. (2010) Crit. Rev. Biotechnol. 30:1.
4. Rollins, B.J. et al. (1989) Mol. Cell. Biol. 9:4687.
5. Jiang, Y. et al. (1990) J. Biol. Chem. 265:18318.
6. Furutani, Y. et al. (1989) Biochem. Biophys. Res. Commun. 159:249.
7. Chakravarty, L. et. al. (1998) J. Biol. Chem. 273:29641.
8. Mortier, A. et al. (2011) Exp. Cell Res. 317:642.
9. Proost, P. et al. (1998) J. Immunol. 160:4034.
10. Ruggiero, P. et al. (2003) Eur. Cytokine Netw. 14:91.
11. Paolini, J.F. et al. (1994) J. Immunol. 153:2704.
12. Ali, S. et al. (2001) Biochem. J. 358:737.
13. Salanga, C.L. and T.M. Handel (2011) Exp. Cell Res. 317:590.
14. Rollins, B.J. et al. (1988) Proc. Natl. Acad. Sci. USA 85:3738.
15. Hosang, K. et al. (1994) Biochem. Biophys. Res. Commun. 199:962.
16. McQuibban, G.A. et al. (2002) Blood 100:1160.
17. Denney, H. et al. (2009) Biochem. Biophys. Res. Commun. 382:341.
18. Feuser, K. et al. (2012) Cytokine. 58:178.
19. Carrillo-de Sauvage, M.A. et al. (2012) PLoS ONE 7:e30762.
20. Paavola, C.D. et al. (1998) J. Biol. Chem. 273:33157.
21. Ancuta, P. et al. (2006) J. Immunol. 176:5760.
22. Fouillet, A. et al. (2012) Brain Res. 1437:115.
23. Rand, M.L. et al. (1996) Am. J. Pathol. 148:855.
24. Zisman, D.A. et al. (1997) J. Clin. Invest. 99:2832.
25. Gong, J.H. et al. (1997) J. Exp. Med. 186:131.
26. Gunn, M.D. et al. (1997) J. Immunol. 158:376.
27. Hughes, P.M. et al. (2002) J. Cereb. Blood Flow Metab. 22:308.
28. Tesch, G.H. et al. (1999) J. Clin. Invest. 103:73.
29. Lu, B. et al. (1998) J. Exp. Med. 187:601.
30. Tesch, G.H. et al. (1999) J. Exp. Med. 190:1813.
31. Kawakami, K. et al. (2001) J. Immunol. 167:6525.
32. Uezu, K. et al. (2004) J. Immunol. 172:7629.

33. Kim, W.J. et al. (2003) Biochem. Biophys. Res. Commun. 310:936.
34. Penido, C. et al. (2003) J. Immunol. 171:6788.
35. Tuaillon, N. et al. (2002) Invest. Ophthalmol. Vis. Sci. 43:1493.
36. Low, Q.E. et al. (2001) Am. J. Pathol. 159:457.
37. Gu, L. et al. (2000) Nature 404:407.
38. Bossink, A.W. et al. (1995) Blood 86:3841.
39. Herfarth, H. et al. (2003) Int. J. Colorectal Dis. 18:401.
40. Tucci, M. et al. (2004) Arthritis Rheum. 50:1842.
41. Henkel, J.S. et al. (2004) Ann. Neurol. 55:221.
42. Scarpini, E. et al. (2002) J. Neurol. Sci. 195:41.
43. Ellingsen, T. et al. (2001) J. Rheumatol. 28:41.
44. Kusano, K.F. et al. (2004) Circ. J. 68:671.
45. Ohta, M. et al. (2003) Int. J. Oncol. 22:773.
46. Ohta, M. et al. (2002) Int. J. Cancer 102:220.
47. Kuratsu, J. et al. (1993) J. Natl. Cancer Inst. 85:1836.
48. Hefler, L. et al. (1999) Br. J. Cancer 81:855.
49. Amann, B. et al. (1998) Br. J. Urol. 82:118.
50. Ueno, T. et al. (2000) Clin. Cancer Res. 6:3282.

PLATE LAYOUT

Use this plate layout to record standards and samples assayed.



产品信息及操作手册

人 CCL2/MCP-1 Valukine™ ELISA 试剂盒

目录号：VAL206

适用于定量检测天然和重组人单核细胞趋化蛋白 1 (MCP-1)/CCL2 的浓度

科研专用，不可用于临床诊断

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有效期详见试剂盒包装标签

Novus 试剂盒确保在你收货日期 3 个月内有效

版本号 202411.1

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I. 背景

MCP-1 又称 CCL2、MCAF 和 TDCF，是一种肝素结合型趋化因子，属于β或 CC 家族趋化因子成员，分子量介于 10-14 kDa。目前，人 CC 家族至少有 26 个成员，大小一般在 8-12 kDa 之间。这些趋化因子大多位于人类第 17 号染色体上，都含有典型的三个β折叠/一个α-螺旋结构 (1-3)。人 MCP-1 以 99 个氨基酸（aa）的前体形式合成，其中包含一个 23 aa 的信号序列和一个 76 aa 的成熟区域。成熟区域包含受体结合和二聚化的 N 端，以及与 GAG 结合的 C 端（2, 7）。MCP-1 会进行 O-糖基化，但不会进行 N-糖基化，糖基化模式的变化导致了其分子量的差异（4, 5, 8, 9）。糖基化程度高的 MCP-1 半衰期延长，而糖基化程度低的 MCP-1 则生物活性增加（10）。MCP-1 以单体形式循环，也有研究表明其可形成二聚体和/或多聚体（11-13）。单体被认为是主要形式，具有完全的趋化活性（11, 12）。二聚体和高级形式被认为是循环细胞与血管内皮细胞之间的联系纽带（13）。小鼠与人 MCP-1 的同源物被称为 JE，其 C 端有一个 49 aa 的延伸。在它们共有的氨基酸序列中，成熟的人 MCP-1 与小鼠 JE 有 57%的序列一致性（14）。成熟的人和猪 MCP-1 氨基酸序列 79%相同（15）。MCP-1 通过蛋白水解过程会产生多种异构体。尽管 MCP-1 不进行 CD26/DPPIV N 端处理，但它会经过 MMP-1, -2, -3 和-9 的 MMP 加工（8, 16, 17）。在 N 端剪切后，会产生了一个保留了一定生物活性的 72 aa 的异构体（aa 28-99）；一个没有活性的 71 aa 的异构体（aa 29-99）和一个具有完全生物活性且经过 C 端处理的异构体（aa 24-92）（9, 16, 17）。多种细胞分泌 MCP-1，包括内皮细胞（EC）、单核细胞、成纤维细胞和血管平滑肌细胞（1, 4, 5）、肥大细胞（18）和星形胶质细胞（19）。

MCP-1 有三种 G 蛋白偶联受体：CCR2、CCR4 和 D6/CCBP2（1, 20-22）。D6 被认为是诱饵受体，可能不会传递信号。CCR2 有两种异构体（A 和 B），它们的胞质尾区不同，表达也不同。异构体 A 存在于 T 细胞和平滑肌细胞中，不能调动钙离子；而异构体 B 存在于单核细胞和活化的 NK 细胞中，且能调动钙。CCR4 在多种造血细胞上表达。MCP-1 最为人知的是其对单核细胞的趋化活性。当 EC 分泌时，它可能与 EC 上的硫酸肝素结合，在那里寡聚化并形成单核细胞上一个 CCR2 的附着点（7,13）。这促进了附着和随后的迁移。当炎症部位的细胞分泌这种物质时，它会诱导细胞向该区域趋化。这种迁移伴随着初始由白细胞衍生的 MMPs 释放，它们通过 ECM 创建了一条通路。然而，这些 MMPs 并不作用于 MCP-1，当到达目标位置后，白细胞会释放新的作用于 MCP-1 的 MMPs（如 MMP-1 和-3），使 MCP-1 失活并为炎症过程提供制动作用（16）。

利用体内动物模型获得的几种不同证据表明，MCP-1 在炎症反应中发挥着重要作用。阻断 MCP-1/JE 的活性可抑制内毒素血症、迟发型超敏反应和炎症性关节炎等动物模型

的症状，而过表达则会增强单核细胞和淋巴细胞的募集（23-26）。相比之下，多项基因敲除研究表明，MCP-1/JE 缺乏的小鼠在包括肺部感染、卒中、血管损伤、肾小管损伤、自身免疫性疾病、葡萄膜炎和伤口愈合等多种模型中，均表现出炎症相关巨噬细胞、单核细胞、自然杀伤（NK）细胞、NKT 细胞和/或γδT 细胞浸润受到抑制（27-36）。MCP-1/JE 缺陷小鼠揭示的其他推测功能还包括在血管生成和初始 T 细胞向 Th2 极化中的作用（36, 37）。人体内 MCP-1 水平升高与败血症、克罗恩病、狼疮性肾炎、肌萎缩侧索硬化症、多发性硬化症、类风湿性关节炎、急性胰腺炎和动脉粥样硬化等疾病相关（38-44）。MCP-1 在多种癌症中也呈上调表达，包括胃癌、食管鳞状细胞癌、恶性胶质瘤以及卵巢癌、胰腺癌、膀胱癌和乳腺癌（45-50）。

II. 概述

A. 检测原理

本实验采用双抗体夹心ELISA法。抗人MCP-1抗体包被于微孔板上，样品和标准品中的人MCP-1会与固定在板上的抗体结合，游离的成分被洗去；加入辣根过氧化酶标记的抗人MCP-1检测抗体进行孵育。洗涤去除未结合的试剂后，加入TMB底物溶液（显色剂）。溶液颜色与结合目标蛋白成正比；加入终止液；用酶标仪测定吸光度。

B. 检测局限

- **仅供科研使用，不可用于体外诊断；**
- 该试剂盒适用于细胞培养上清样本，人血清样本、人血浆样本和人尿液样本；
- 请在试剂盒有效期内使用；
- 不同试剂盒及不同批号试剂盒的组分不能混用；
- 样本值若大于标准曲线的最高值，应将样本用标准品稀释液（1×）/标准品稀释液RD6Q 稀释并重复测定。
- 检测结果的不同可由多种因素引起，包括实验人员的操作、移液器的使用方式、洗板技术、反应时间或温度、试剂盒的效期等。

III. 优势

A. 精确度

板内精确度（同一板内不同孔间的精确度）

已知浓度的三个样本，在同一板内分别检测20次，以确定板内精确度。

板间精确度（不同板之间的精确度）

已知浓度的三个样本，在不同板间分别检测20次，以确定板间精确度。

细胞培养上清/尿液试验

	板内精确度			板间精确度		
样本	1	2	3	1	2	3
平均值 (pg/mL)	78.2	360	1079	76.0	360	1086
标准差	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

血清/血浆试验

	板内精确度			板间精确度		
样本	1	2	3	1	2	3
平均值 (pg/mL)	76.7	364	1121	74.2	352	1076
标准差	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

B. 回收率

不同类型样本中掺入检测范围内不同水平的人MCP-1，测定其回收率。

样本类型	平均回收率 (%)	范围 (%)
细胞培养基 (n=5)	96	88-107
人血清* (n=5)	103	92-113
人 EDTA 血浆* (n=5)	96	92-102
人肝素血浆* (n=5)	102	94-114
人柠檬酸钠血浆*（n=5）	100	94-107
人尿液* (n=5)	92	85-100

*样品在分析前按照样品制备部分的指示进行稀释。

C. 灵敏度

34次试验评估表明，人MCP-1的最低可检测剂量（MDD）范围为0.57-10.0 pg/mL。平均MDD为1.7 pg/mL。

MDD是根据20个重复的零标准品孔的吸光度值的平均值加两倍标准差计算得到的相对应浓度。

D. 校正

该免疫测定法以R&D Systems生产的高纯度的大肠杆菌表达的重组人MCP-1校正。本试剂盒评估了 NIBSC MCP-1 non-WHO 标准物质 92/794（源自人rDNA）。

92/794 标准物质的剂量反应曲线与 Valukine 标准曲线相似。要将 Valukine 人 MCP-1 试剂盒获得的样本值转换为近似 NIBSC 92/794 单位，请使用下面的公式。

NIBSC (92/794) 近似值（U/mL）= 0.002 x Valukine 人 MCP-1 值（pg/mL）

E. 线性

不同的样本中含有或掺入高浓度的人MCP-1，然后用标准品稀释液（1×）/标准品稀释液 RD6Q 将样本稀释到检测范围内，测定其线性。

稀释倍数		细胞培养基 (n=5)	人血清* (n=5)	人EDTA血浆* (n=5)	人肝素血浆* (n=5)	人柠檬酸钠血浆* (n=5)	人尿液* （n=5）
1:2	平均值/期待值（%）	102	99	96	98	95	95
	范围 (%)	98-106	97-101	92-99	89-103	91-97	89-108
1:4	平均值/期待值（%）	102	97	96	96	95	92
	范围 (%)	97-108	91-103	90-106	88-102	91-100	87-102
1:8	平均值/期待值（%）	103	97	98	96	96	91
	范围 (%)	96-109	90-102	92-108	89-103	89-106	88-97
1:16	平均值/期待值（%）	101	95	94	98	94	90
	范围 (%)	93-109	84-106	87-98	87-107	89-100	83-99

*样品在分析前按照样品制备部分的指示进行稀释。

F. 样本预值

人血清/人血浆/人尿液–在本试验中评估了表面健康志愿者样本中是否存在人 MCP-1。本研究中使用的供体没有病史。报告的尿液值为实际值，未对肌酐含量进行标准化。

样本类型	平均值 (pg/mL)	范围 (pg/mL)
人血清 (n=37)	370	200-722
人EDTA血浆 (n=37)	153	72-295
人枸橼酸钠血浆 (n=37)	196	134-436
人肝素血浆 (n=37)	242	113-340
人唾液 (n=37)	211	42-410

细胞培养上清–人外周血白细胞培养在含10%胎牛血清的RPMI 1640 培养基中。细胞在不刺激或用10 μg/mL PHA刺激，培养2天和5天，取出等量细胞培养上清，检测人MCP-1的水平。

条件	2 天 (pg/mL)	5 天 (pg/mL)
未刺激	647	1785
刺激	67225	70000

G. 特异性

检测方法识别天然和重组人MCP-1。

以下列出的因子在标准品稀释液（1×）/标准品稀释液 RD6Q 中以 1.0 ng/mL， 10 ng/mL 和 50 ng/mL 的浓度制备，并进行交叉反应性测定。将下列的因子以1.0 ng/mL，10 ng/mL 和 50 ng/mL 的浓度掺入中间范围的重组人MCP-1对照品中，来检测对人MCP-1的干扰。未观察到明显的交叉反应或干扰。

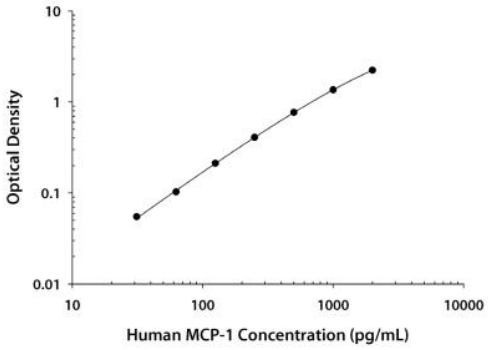
Recombinant human：		**Recombinant mouse：**
GROα	MCP-3	JE/MCP-1
GROβ	MIP-1α	MIP-1α
GROγ	MIP-1β	MIP-1β
IL-8	RANTES	**Recombinant rat：**
MCP-2		JE/MCP-1

IV. 实验

标准曲线实例

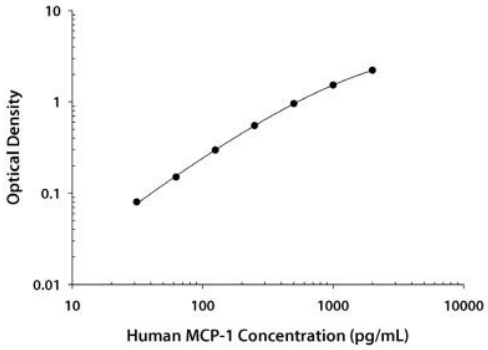
该标准曲线数据仅供参考，每次实验应绘制其对应的标准曲线。

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

V. 试剂盒组成及储存

A. 试剂盒组成

组成	描述	规格
Human MCP-1 Microplate	包被抗人MCP-1抗体的96孔聚苯乙烯板，8孔× 12条	1块板
Human MCP-1 Conjugate	酶标检测抗人MCP-1抗体	1瓶
Human MCP-1 Standard	人MCP-1标准品（冻干），参考瓶身标签进行重溶	1瓶
Assay Diluent RD1-83	检测液	1瓶
Calibrator Diluent Concentrate (5×)/ RD5L	浓缩的标准品稀释液（5×）用于稀释标准品和样本(用于细胞培养上清/尿液样本)	1瓶
Calibrator Diluent RD6Q	标准品稀释液用于稀释标准品和样本（用于血清/血浆样本）	2瓶
Wash Buffer Concentrate (25×)	浓缩洗涤缓冲液（25×）	1瓶
TMB Substrate	TMB ELISA底物溶液/TMB底物溶液	2瓶
Stop Solution	终止液	1瓶
Plate Sealers	封板膜	3张

B. 试剂盒储存

<table>
<tr><td>未开封试剂盒</td><td colspan="2">2-8°C储存；请在试剂盒有效期内使用</td></tr>
<tr><td rowspan="8">已打开，稀释或重溶的试剂</td><td>洗涤液（1×）</td><td rowspan="6">2-8°C储存，最多30天*</td></tr>
<tr><td>终止液</td></tr>
<tr><td>检测液RD1-83</td></tr>
<tr><td>标准品稀释液RD6Q</td></tr>
<tr><td>酶标检测抗体</td></tr>
<tr><td>TMB底物溶液</td></tr>
<tr><td>标准品</td><td>分装，-20℃以下冰箱储存最多30天*；避免反复冻融。</td></tr>
<tr><td>浓缩标准品稀释液（5×）/ RD5L</td><td>2-8℃储存，最多30天*
请每次使用新鲜配制的1×标准品稀释液，多余的丢弃。</td></tr>
<tr><td>包被的微孔板条</td><td>将未用的板条放回带有干燥剂的铝箔袋内，密封；2-8 °C储存，最多30天*</td></tr>
</table>

*必须在试剂盒有效期内

C. 实验所需自备试验器材

- 酶标仪（可测量450 nm检测波长的吸收值及540 nm或570 nm校正波长的吸收值）
- 高精度加液器及一次性吸头
- 蒸馏水或去离子水
- 洗瓶（喷瓶）、多通道洗板器或自动洗板机
- 100 mL和500 mL量筒
- 用于稀释标准品和样本的聚丙烯管

D. 注意事项

- 试剂盒中的一些组分含有防腐剂，可能引起皮肤过敏反应，避免吸入。
- 试剂盒的某些成分含有叠氮化钠，可能会与铅和镉发生反应。在铜管中会形成爆炸性金属叠氮化物。处理时要大量水冲洗。
- 试剂盒中的终止液是酸性溶液，使用时请做好眼睛、手、面部及衣服的防护。

VI. 实验前准备

A. 样品收集及储存

以下列出的样品收集和储存条件仅作为一般指南。样品稳定性尚未评估。

细胞培养上清液 – 为防止检测前细胞培养上清中 MCP-1 的损失，建议培养基中至少添加2%的胎牛血清。通过离心去除颗粒物，并立即检测或分装并储存在≤ -20°C，避免反复冻融。样品可能需要用标准品稀释液（1×）稀释。

血清 - 使用血清分离管(SST)，让样本在室温下凝固30分钟，然后在1000 × g 离心15分钟。立即检测或分装并储存在≤ -20 °C。避免反复冻融。样本可能需要用标准品稀释液 RD6Q 稀释。

血浆 - 使用EDTA、肝素和枸橼酸钠作为抗凝剂，然后1000 × g离心15分钟，需在30分钟内收集血浆样本，立即检测或分装并储存在≤ -20 °C。避免反复冻融。样品可能需要用标准品稀释液 RD6Q 稀释。

尿液 - 无菌收集一天中的第一次尿液（中段），直接排入无菌容器中。离心去除颗粒物质。立即检测或分装并储存在 ≤ -20 °C。避免反复冻融。样品可能需要用标准品稀释液（1×）稀释。

B. 样品准备

警告：必须使用聚丙烯材质管，请不要使用玻璃材质管。

所有人血清样本和人血浆样本建议 2 倍稀释。建议的 2 倍稀释量为：250 µL 的样本+250 µL 的标准品稀释液 RD6Q。最佳稀释倍数由最终用户确定。

所有人尿液样本建议进行 2 倍稀释。建议的 2 倍稀释量为：250 µL 的样本+250 µL 的标准品稀释液（1×）。最佳稀释倍数由最终用户确定。

C. 检测前准备工作

使用前请将所有试剂放置于室温。

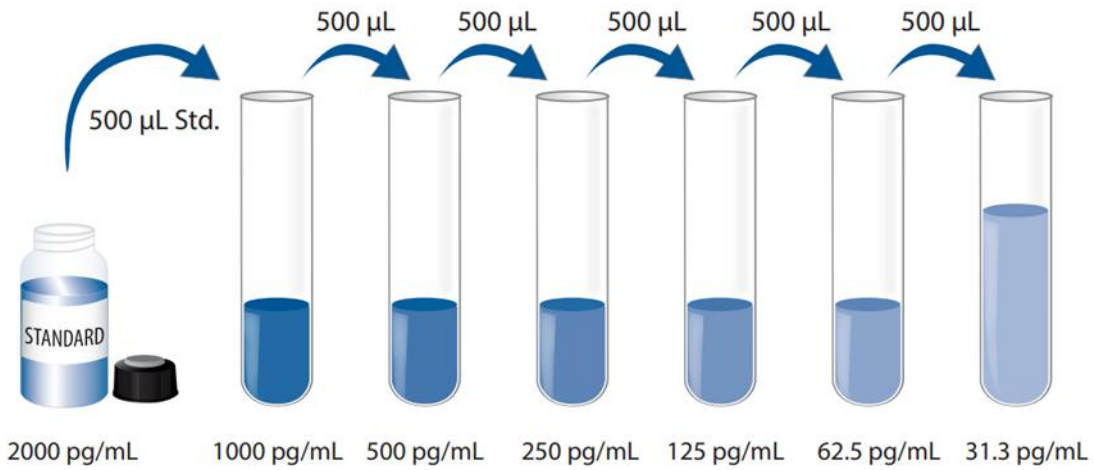
洗涤液（1×）：从冰箱中取出的浓缩洗涤液可能有结晶，属于正常现象；放置室温，轻摇混匀，待结晶完全溶解后再配制洗涤液。可将20 mL浓缩洗涤液（25×）用蒸馏水或去离子水稀释配制成500 mL工作浓度的洗涤液（1×）。

标准品稀释液（1×）：使用去离子水或蒸馏水制备标准品稀释液（1×）。

人MCP-1标准品：重溶体积请参考瓶身标签*，用标准品稀释液（1×）（用于细胞培养上清/尿液样本）或标准品稀释液 RD6Q （用于血清/血浆样本）重溶人MCP-1标准品，得到浓度为 2000 pg/mL标准品储备母液。轻轻震摇至少15分钟，其充分溶解。

*如有疑问，请咨询我们的技术支持。

使用聚丙烯管。将500 µL标准品稀释液（1×）（用于细胞培养上清/尿液样品）或标准品稀释液 RD6Q（用于血清/血浆样品）移入每管中。将标准品母液参照下图做系列稀释。每管须充分混匀后再移液到下一管。没有稀释的标准品母液可用作标准曲线最高点（2000 pg/mL），标准品稀释液（1×）或标准品稀释液 RD6Q可用作标准曲线零点（0 pg/mL）。



D. 技术小提示

- 当混合或重溶蛋白液时，尽量避免起沫；
- 为了避免交叉污染，配制不同浓度标准品、上样、加不同试剂都需要更换枪头。另外不同试剂请分别使用不同的移液槽；
- 建议15分钟内完成一块板的上样；
- 每次孵育时，正确使用封板膜可保证结果的准确性；
- TMB底物溶液在上板前应为无色，请避光保存；加入微孔板后，将由无色变成不同深度的蓝色；
- 终止液上板顺序应同TMB底物溶液上板顺序一致；加入终止液后，孔内颜色由蓝变黄；若孔内有绿色，则表明孔内液体未混匀请充分混合；

VII.操作步骤

使用前将所有试剂和样品平衡至室温。建议对所有标准品和样品进行复孔检测。

1. 按照上一节的说明，准备好所有需要的试剂和标准品。
2. 从已平衡至室温的密封袋中取出微孔板，未用的板条请放回铝箔袋内，重新封口。
3. **仅用于血清/血浆样品：**向每个孔中加入50 µL的检测液RD1-83。*检测液RD1-83中可能含有结晶沉淀。使用前和使用过程中请充分混合。*
4. 分别将不同浓度标准品和实验样本加入相应孔中，每孔200 µL。用封板膜封住反应孔，**在室温下孵育2小时**。说明书提供了一张96孔模板图，可用于记录标准品和试验样本的板内位置。
5. 将板内液体吸去，使用洗瓶、多通道洗板器或自动洗板机洗板。每孔加洗涤液400 µL，然后将板内洗涤液吸去。重复操作2次，共洗3次。每次洗板尽量吸去残留液体会有助于得到好的实验结果。最后一次洗板结束，请将板内所有液体吸干或将板倒置，在吸水纸拍干所有残留液体。
6. 在每个微孔内加入200 µL人MCP-1酶标检测抗体。用封板膜封住反应孔，**培养上清/尿液样本：室温孵育1小时。培养血清/血浆样品：室温培养2小时**。
7. 重复第5步洗板操作。
8. 在每个微孔内加入200 µL TMB底物溶液，**室温孵育30分钟。注意避光**。
9. 在每个微孔内加入50 µL终止液，请轻拍微孔板，使溶液混合均匀。
10. 加入终止液后10分钟内，使用酶标仪测量450 nm的吸光度值，设定540 nm或570 nm作为校正波长。如果波长校正不可用，以450 nm的读数减去540 nm或570 nm的读数。这种减法将校正酶标板上的光学缺陷。没有校正而直接在450 nm处进行的读数可能会更高且更不准确。
11. 计算结果：将每个标准品和样品的复孔吸光值取平均值，然后减去零标准品平均OD值（O.D.）使用计算机软件作四参数逻辑（4-PL）曲线拟合创建标准曲线。另一替代方法是，通过绘制y轴上每个标准品的平均吸光值与x轴上的浓度来构建标准曲线，并通过图上的点绘制最佳拟合曲线。数据可以通过绘制人MCP-1浓度的对数与O.D.的对数来线性化，并且最佳拟合线可以通过回归分析来确定。该程序将产生足够但不太精确的数据拟合。

    如果样品被稀释，从标准曲线读取的浓度必须乘以稀释倍数。

VIII. 参考文献

1. Deshmane, S.L. et al. (2009) J. Interferon Cytokine Res. 29:313.
2. van Coillie, E. et al. (1999) Cytokine Growth Factor Res. 10:61.
3. Sharma, M. et al. (2010) Crit. Rev. Biotechnol. 30:1.
4. Rollins, B.J. et al. (1989) Mol. Cell. Biol. 9:4687.
5. Jiang, Y. et al. (1990) J. Biol. Chem. 265:18318.
6. Furutani, Y. et al. (1989) Biochem. Biophys. Res. Commun. 159:249.
7. Chakravarty, L. et. al. (1998) J. Biol. Chem. 273:29641.
8. Mortier, A. et al. (2011) Exp. Cell Res. 317:642.
9. Proost, P. et al. (1998) J. Immunol. 160:4034.
10. Ruggiero, P. et al. (2003) Eur. Cytokine Netw. 14:91.
11. Paolini, J.F. et al. (1994) J. Immunol. 153:2704.
12. Ali, S. et al. (2001) Biochem. J. 358:737.
13. Salanga, C.L. and T.M. Handel (2011) Exp. Cell Res. 317:590.
14. Rollins, B.J. et al. (1988) Proc. Natl. Acad. Sci. USA 85:3738.
15. Hosang, K. et al. (1994) Biochem. Biophys. Res. Commun. 199:962.
16. McQuibban, G.A. et al. (2002) Blood 100:1160.
17. Denney, H. et al. (2009) Biochem. Biophys. Res. Commun. 382:341.
18. Feuser, K. et al. (2012) Cytokine. 58:178.
19. Carrillo-de Sauvage, M.A. et al. (2012) PLoS ONE 7:e30762.
20. Paavola, C.D. et al. (1998) J. Biol. Chem. 273:33157.
21. Ancuta, P. et al. (2006) J. Immunol. 176:5760.
22. Fouillet, A. et al. (2012) Brain Res. 1437:115.
23. Rand, M.L. et al. (1996) Am. J. Pathol. 148:855.
24. Zisman, D.A. et al. (1997) J. Clin. Invest. 99:2832.
25. Gong, J.H. et al. (1997) J. Exp. Med. 186:131.
26. Gunn, M.D. et al. (1997) J. Immunol. 158:376.
27. Hughes, P.M. et al. (2002) J. Cereb. Blood Flow Metab. 22:308.
28. Tesch, G.H. et al. (1999) J. Clin. Invest. 103:73.
29. Lu, B. et al. (1998) J. Exp. Med. 187:601.
30. Tesch, G.H. et al. (1999) J. Exp. Med. 190:1813.
31. Kawakami, K. et al. (2001) J. Immunol. 167:6525.
32. Uezu, K. et al. (2004) J. Immunol. 172:7629.

33. Kim, W.J. et al. (2003) Biochem. Biophys. Res. Commun. 310:936.
34. Penido, C. et al. (2003) J. Immunol. 171:6788.
35. Tuaillon, N. et al. (2002) Invest. Ophthalmol. Vis. Sci. 43:1493.
36. Low, Q.E. et al. (2001) Am. J. Pathol. 159:457.
37. Gu, L. et al. (2000) Nature 404:407.
38. Bossink, A.W. et al. (1995) Blood 86:3841.
39. Herfarth, H. et al. (2003) Int. J. Colorectal Dis. 18:401.
40. Tucci, M. et al. (2004) Arthritis Rheum. 50:1842.
41. Henkel, J.S. et al. (2004) Ann. Neurol. 55:221.
42. Scarpini, E. et al. (2002) J. Neurol. Sci. 195:41.
43. Ellingsen, T. et al. (2001) J. Rheumatol. 28:41.
44. Kusano, K.F. et al. (2004) Circ. J. 68:671.
45. Ohta, M. et al. (2003) Int. J. Oncol. 22:773.
46. Ohta, M. et al. (2002) Int. J. Cancer 102:220.
47. Kuratsu, J. et al. (1993) J. Natl. Cancer Inst. 85:1836.
48. Hefler, L. et al. (1999) Br. J. Cancer 81:855.
49. Amann, B. et al. (1998) Br. J. Urol. 82:118.
50. Ueno, T. et al. (2000) Clin. Cancer Res. 6:3282.

96 孔模板图

请使用 96 孔模板图来记录标准品及样本在板内的位置