# PRODUCT INFORMATION & MANUAL

## Mouse CCL2/JE/MCP-1 Valukine™ ELISA

## Catalog Number: VAL636

For the quantitative determination of natural and recombinant mouse Monocyte Chemotactic Protein 1 (MCP-1) /CCL2/JE 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 202410.1

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# I. BACKGROUND

The mouse JE gene was originally described as a platelet-derived growth factor-inducible gene in mouse fibroblasts (1). The protein encoded by mouse JE was found to belong to the large CC chemokine family of inflammatory and immunoregulatory cytokines. Among CC chemokine family members, JE is functionally and structurally most closely related to the MCP/eotaxin subfamily of proteins. Within this MCP/eotaxin subfamily, five human (MCP-1, 2, 3, 4 and eotaxin) and four mouse (JE/MCP-1, MARC/MCP-3, MCP-5, and eotaxin) proteins have been identified (1-3). At the amino acid (aa) sequence level, mature human MCP-1 shows 55%, 59%, and 66% identity with the analogous regions of mouse JE, MARC, and MCP-5, respectively (1-3). Although JE has been presumed to be the mouse homolog of human MCP-1 (3-6), the more recently isolated mouse MCP-5 is actually more homologous and may be considered to be a second human MCP-1 homolog (7).

Mouse MCP-1 cDNA encodes a 148 aa residue precursor protein with a predicted 23 aa residue signal peptide that is cleaved to generate a putative mature protein of 125 aa residues (1-3). Compared to mature human MCP-1, mouse MCP-1 has a 49 aa residue carboxy-terminal extension that is rich in serine and threonine residues. Recombinant MCP-1 expressed in CHO cells (2) as well as natural MCP-1 purified from mouse astrocytes (4, 10) and a mouse thymic epithelial cell line (8), were shown to be approximately 30 kDa glycoproteins with multiple O-linked oligosaccharide chains added to the 49 aa residue C-terminal domain. Nonetheless, the natural form of MCP-1 produced by virus-stimulated mouse L929 fibroblasts occurs as a non-glycosylated 7-8 kDa protein that lacks the C-terminal domain (12). The carboxy-terminal domain has been found not to be required for mouse MCP-1 activity. Besides fibroblasts, astrocytes and epithelial cells, mouse MCP-1 has been found to be expressed in macrophages (4, 9), mast cells (7), endothelial cells (7), osteoblasts and ameloblasts (11). The expression of mouse MCP-1 is induced after stimulation with inflammatory stimuli including viruses, LPS, and cytokines such as TNF-α, IL-1, IFN-γ, and PDGF (1, 3, 4, 12, 13).

Mouse MCP-1 is a potent chemoattractant for monocytes/macrophages and lymphocytes (3, 4, 13, 17). It has also been shown to be involved in the regulation of Th1/Th2 lymphocyte differentiation, enhancing Th2 development by increasing IL-4 production and inhibiting IL-12 production (18-21). The activities of mouse MCP-1 have been shown to be mediated by the mouse CC chemokine receptor CCR2, a G protein-coupled, seven transmembrane domain receptor (6, 14, 15). Mouse CCR2 cDNA encodes a 373 aa residue protein that shows the highest (80%) overall identity at the aa sequence level with the human MCP-1 receptor, CCR2B (14-16). The gene for mouse CCR2 has been mapped to mouse chromosome 9, in close proximity with mouse CCR1 and CCR3. High levels of mouse CCR2 expression have been detected in monocytes/macrophages.

# II. OVERVIEW

## A. PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. An antibody specific for mouse MCP-1 has been pre-coated onto a microplate. Standards, control and samples are pipetted into the wells and any mouse MCP-1 present is bound by the immobilized antibody. After washing away any unbound substances, an enzyme-linked antibody specific for mouse 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 mouse 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 ROCEDURES.**
- This kit is suitable for cell culture supernates, tissue culture supernates, tissue lysates, mouse plasma and mouse serum.
- 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 RD5-3 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.

| | Intra-assay Precision | | | Inter-assay Precision | | |
|---|---|---|---|---|---|---|
| Sample | 1 | 2 | 3 | 1 | 2 | 3 |
| Mean (pg/mL) | 36.7 | 51.8 | 149 | 37.8 | 60.0 | 155 |
| Standard Deviation | 1.45 | 1.40 | 3.66 | 2.85 | 4.37 | 7.86 |
| CV% | 4.0 | 2.7 | 2.5 | 7.5 | 7.3 | 5.1 |

## B. RECOVERY

The recovery of mouse 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=4) | 105 | 95-112 |
| Lysis buffer (n=4) | 88 | 79-101 |
| Mouse serum (n=4) | 91 | 83-100 |
| Mouse EDTA plasma (n=4) | 84 | 75-93 |
| Mouse heparin plasma (n=4) | 93 | 80-101 |

## C. SENSITIVITY

Twenty-two assays were evaluated and the minimum detectable dose (MDD) of mouse MCP-1 ranged from 0.151-0.666 pg/mL. The mean MDD was 0.304 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 mouse MCP-1 produced at R&D Systems.

## E. LINEARITY

To assess the linearity of the assay, different samples were containing or spiked with high concentrations of mouse MCP-1 and diluted with Calibrator Diluent RD5-3 to produce samples with values within the dynamic range of the assay.

| | | **Cell culture supernates* (n=4)** | **Lysis buffer (n=4)** | **Mouse serum (n=4)** | **Mouse EDTA plasma (n=4)** | **Mouse heparin plasma (n=4)** |
|---|---|---|---|---|---|---|
| 1:2 | Average % of Expected | 105 | 99 | 96 | 96 | 107 |
| | Range (%) | 102-107 | 95-108 | 94-99 | 93-104 | 104-109 |
| 1:4 | Average % of Expected | 110 | 102 | 101 | 102 | 111 |
| | Range (%) | 106-116 | 94-107 | 97-109 | 101-105 | 104-116 |
| 1:8 | Average % of Expected | 109 | 109 | 105 | 107 | 104 |
| | Range (%) | 105-113 | 103-116 | 100-116 | 100-110 | 98-110 |
| 1:16 | Average % of Expected | 115 | 119 | 106 | 107 | 108 |
| | Range (%) | 107-125 | 113-124 | 101-116 | 104-112 | 102-119 |

* Samples were diluted prior to assay.

## F. SAMPLE VALUES

**Mouse serum/plasma -** Samples were evaluated for detectable levels of mouse MCP-1 in this assay.

| | Mean (pg/mL) | Range (pg/mL) | Standard Deviation (pg/mL) |
|---|---|---|---|
| Mouse serum (n=10) | 114 | 64.5-216 | 44.7 |
| Mouse EDTA plasma (n=10) | 76.6 | 42.5-116 | 23.8 |
| Mouse heparin plasma (n=10) | 96.6 | 72.2-154 | 25.9 |

**Cell Culture Supernates:**

C2C12 mouse myoblast cells were cultured in DMEM and supplemented with 10% fetal bovine serum until confluent. The cells were then washed with PBS and allowed to grow in DMEM supplemented with 5% equine serum for 5-7 days with media changes every other day. Once the cells were 50% differentiated, they were untreated or treated with lipopolysaccharide (LPS) for 3 days. Aliquots of the cell culture supernates were removed, assayed for levels of mouse MCP-1, and measured 2359 pg/mL and 11993 pg/mL, respectively.

J774A.1 mouse reticulum cell sarcoma macrophage cells were cultured in RPMI and supplemented with 10% fetal bovine and 2 mM L-glutamine. Cells were untreated or treated with LPS for 3 days. Aliquots of the cell culture supernates were removed, assayed for levels of mouse MCP-1, and measured 8430 pg/mL and 34717 pg/mL, respectively.

**Tissue Culture Supernates -** Mouse splenocytes were homogenized and cultured in RPMI 1640 supplemented with 10% fetal bovine serum, 2 mM L-glutamine, 100 U/mL penicillin, and 100 µg/mL streptomycin sulfate and incubated for 2 days. An aliquot of the cell culture supernate was removed, assayed for mouse MCP-1 and measured 24.7 pg/mL.

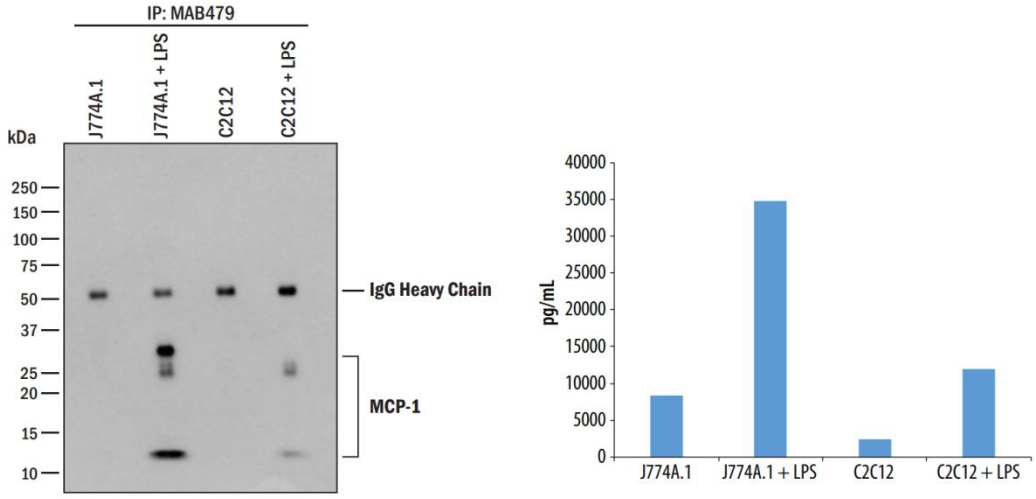
**Tissue Lysates -** Mouse lung tissue was rinsed with PBS and placed on ice. PBS was poured off and 10 mL of ice-cold lysis buffer with protease inhibitors was added. Tissue was homogenized with a polytron homogenizer with 2 x 5 second bursts on ice. Samples were centrifuged at 2000 rpm for 5 minutes. Quantitation of sample protein concentration using a total protein assay is recommended. 50 µg of the tissue lysate was removed, assayed for mouse MCP-1, and measured 26.8 pg/mL.

## G. SPECIFICITY

This assay recognizes natural and recombinant mouse MCP-1.

The factors listed below were prepared at 50 ng/mL in Calibrator Diluent RD5-3 and assayed for cross-reactivity. Preparations of the following factors prepared at 50 ng/mL in a mid-range mouse MCP-1 control were assayed for interference. No significant cross-reactivity or interference was observed.

| **Recombinant mouse:** | | **Other recombinant:** |
|---|---|---|
| Eotaxin | MIP-1α | human MCP-1 |
| MARC | MIP-1β | rat MCP-1 |
| MCP-2 | MIP-1γ | |
| MCP-5 | MIP-3α | |

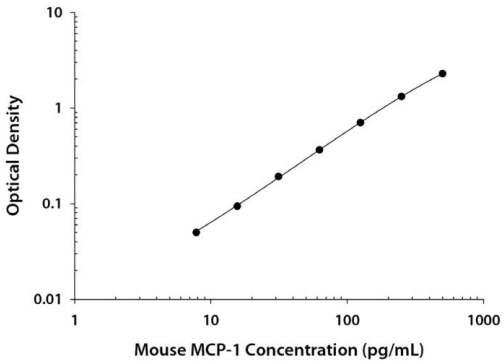



Conditioned media from J774A.1 and C2C12 cells left untreated or treated with LPS for 72 hours were analyzed by immunoprecipitation Western Blot and Valukine ELISA. Conditioned media was immunoprecipitated using Rat anti-Mouse CCL2/JE/MCP-1 (R&D Systems®, Catalog # MAB479). For Western Blot, samples were resolved under reducing SDS-PAGE conditions, transferred to a PVDF membrane, and immunoblotted with Goat anti-Mouse CCL2/JE/MCP-1 (R&D Systems®, Catalog # AF-479-NA). The immunoprecipitation Western Blot shows a direct correlation with ELISA value for these samples.

# IV. EXPERIMENT

## EXAMPLE STANDARD

The standard curve is provided for demonstration only. A standard curve should be generated for each set of samples assayed.




| (pg/mL) | O.D. | Average | Corrected |
|---|---|---|---|
| 0 | 0.007<br>0.007 | 0.007 | — |
| 7.81 | 0.056<br>0.057 | 0.057 | 0.050 |
| 15.6 | 0.100<br>0.101 | 0.101 | 0.094 |
| 31.3 | 0.196<br>0.202 | 0.199 | 0.192 |
| 62.5 | 0.365<br>0.379 | 0.372 | 0.365 |
| 125 | 0.702<br>0.715 | 0.709 | 0.702 |
| 250 | 1.320<br>1.332 | 1.326 | 1.319 |
| 500 | 2.274<br>2.317 | 2.296 | 2.289 |

# V. KIT COMPONENTS AND STORAGE

## A. MATERIALS PROVIDED

| Parts | Description | Size |
|---|---|---|
| Mouse MCP-1 Microplate | 96 well polystyrene microplate (12 strips of 8 wells) coated with an antibody against mouse MCP-1. | 1 plate |
| Mouse MCP-1 Conjugate | An antibody specific for mouse MCP-1 conjugated to horseradish peroxidase. | 1 vial |
| Mouse MCP-1 Standard | Recombinant mouse MCP-1 in a buffered protein base; lyophilized. Refer to the vial label for reconstitution volume. | 2 vials |
| Mouse MCP-1 Control | Recombinant mouse MCP-1 in a buffered protein base; lyophilized. The assay value of the control should be within the range specified on the label. | 2 vials |
| Assay Diluent RD1W | A buffered protein base. | 1 vial |
| Calibrator Diluent RD5-3 | A buffered protein base used to dilute standard and samples. | 1 vial |
| Wash Buffer Concentrate (25×) | A 25× concentrated solution of buffered surfactant. | 1 vial |
| TMB Substrate | TMB ELISA Substrate Solution/TMB Substrate Solution. | 2 vial |
| Stop Solution | Diluted hydrochloric 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>Assay Diluent RD1W</td></tr>
<tr><td>Calibrator Diluent RD5-3</td></tr>
<tr><td>Stop Solution</td></tr>
<tr><td>Conjugate</td></tr>
<tr><td>TMB Substrate</td><td rowspan="2">Use a new standard and control for each assay. Discard after use.</td></tr>
<tr><td>Control</td></tr>
<tr><td>Standard</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.
- 500 mL graduated cylinder.
- Test tubes for dilution of standards and samples.

## D. SUPPLIES REQUIRED FOR TISSUE LYSATE SAMPLES

- Cell Lysis Buffer 2 (R&D Systems®, Catalog # 895347), Cell Lysis Buffer 3 (R&D Systems®, Catalog # 895366), Lysis Buffer 16 (R&D Systems®, Catalog # 895935), or Lysis Buffer 17(R&D Systems®, Catalog # 895943).
- PBS

## E. PRECAUTION

- Some components in this kit contain a preservative which may cause an allergic skin reaction. Avoid breathing mist.
- 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 -** 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 RD5-3.

**Tissue Culture Supernates -** Tissues must be prepared prior to assay as described in the Sample Values section. Samples may require dilution with Calibrator Diluent RD5-3.

**Tissue Lysates -** Lysates were prepared prior to assay as described in the Sample Values section. Samples may require dilution with Calibrator Diluent RD5-3.

**Serum -** Allow blood samples to clot for 2 hours at room temperature before centrifuging for 20 minutes at 2000 × 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 RD5-3.

**Plasma -** Collect plasma using EDTA or heparin as an anticoagulant. Centrifuge for 20 minutes at 2000 x 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 RD5-3.

***Note:*** *Citrate plasma has not been validated for use in this assay.*

## B. REAGENT PREPARATION

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

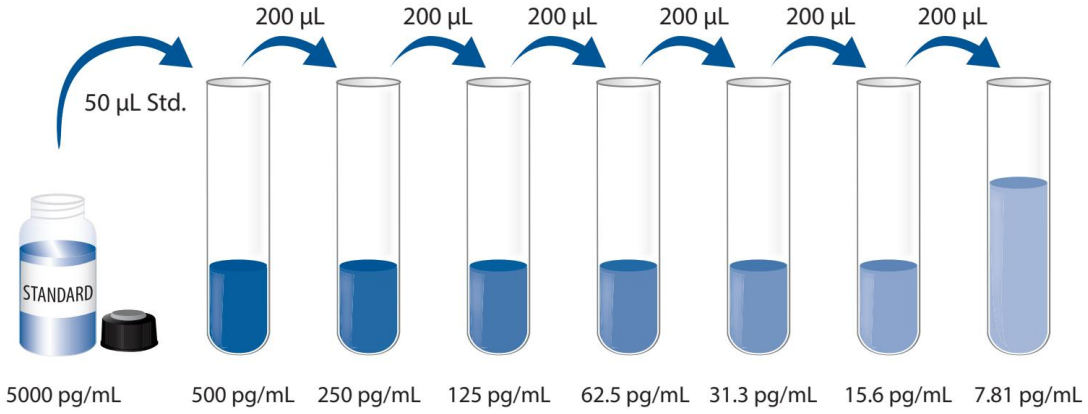
**Mouse MCP-1 Control -** Reconstitute the control with 1.0 mL of deionized or distilled water. Mix thoroughly. Assay the control undiluted.

**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×).

**Mouse MCP-1 Standard- Refer to the vial label for the reconstitution volume*** Reconstitute the Mouse MCP-1 Standard with deionized or distilled water. Do not substitute other diluents. This reconstitution produces a stock solution of 5000 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.

**Pipette 450 µL of Calibrator Diluent RD5-3 into the 500 pg/mL tube. Pipette 200 µL into each of the remaining tubes.** Use the stock solution to produce a dilution series (below). Mix each tube thoroughly before the next transfer. The 500 pg/mL standard serves as the high standard. The Calibrator Diluent RD5-3 serves as the zero standard (0 pg/mL).




## C. 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 reagents and samples to room temperature before use. It is recommended that all samples, control and standards be assayed in duplicate.**

1. Prepare all reagents, working standards, control 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. Add 50 µL of Assay Diluent RD1W to each well.
4. Add 50 µL of standard, control 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 three times for a total of four washes. Wash by filling each well with Wash Buffer (400 µL) using a squirt bottle, manifold dispenser, or autowasher. Complete removal of liquid at each step is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against clean paper towels.
6. Add 100 µL of Mouse MCP-1 Conjugate to each well. Cover with a new adhesive strip. **Incubate for 2 hours at room temperature.**
7. Repeat the aspiration/wash as in step 5.
8. Add 100 µL of TMB Substrate to each well. **Incubate for 30 minutes at room temperature. Protect from light**.
9. Add 100 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, 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 mouse 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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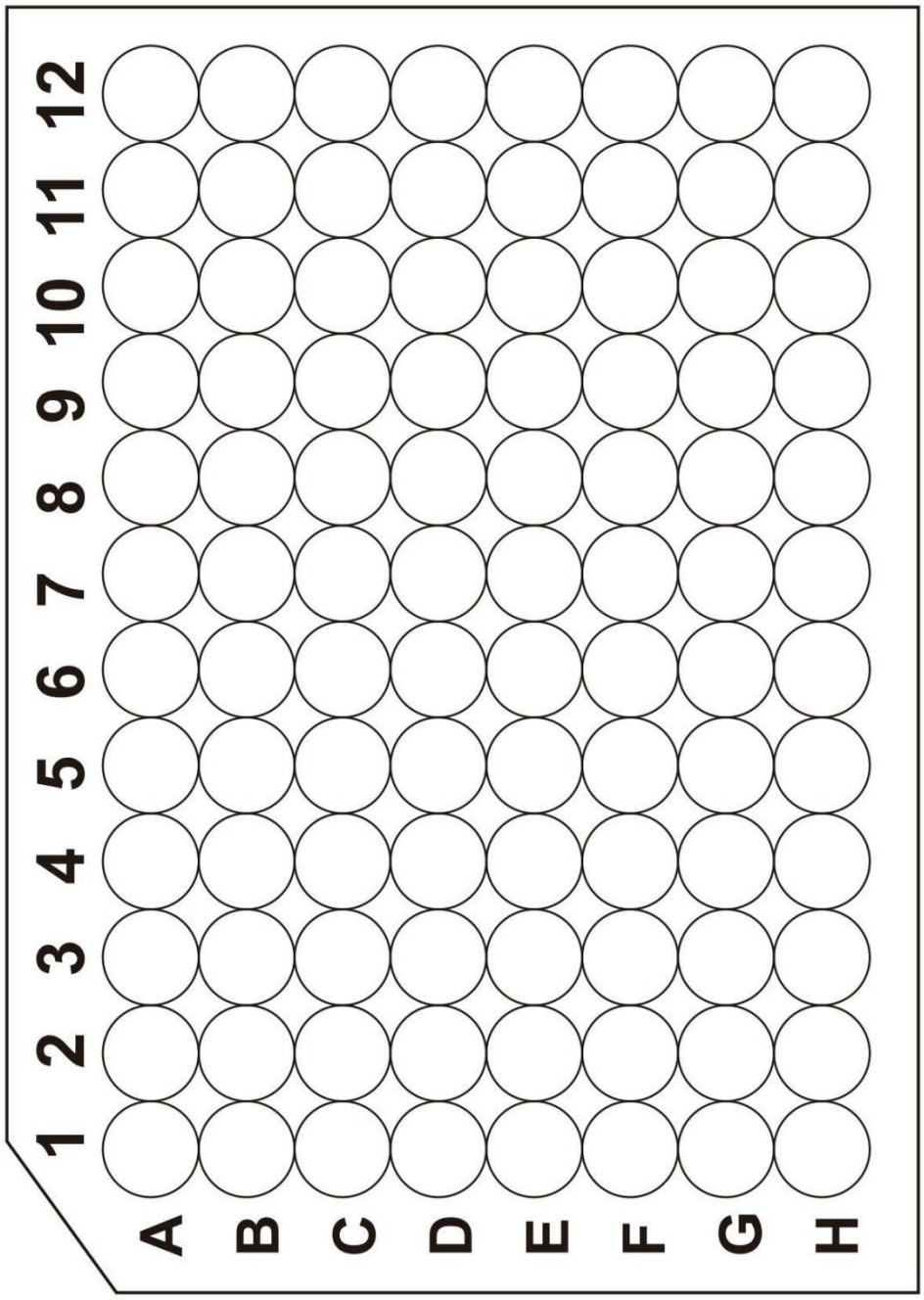
## PLATE LAYOUT

Use this plate layout to record standards and samples assayed.



# 产品信息及操作手册

## 小鼠 CCL2/JE/MCP-1 Valukine™ ELISA 试剂盒

## 目录号：VAL636

适用于定量检测天然和重组小鼠单核细胞趋化蛋白 1 (MCP-1) / CCL2/JE 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 202410.1

# 目录

# I. 背景

小鼠 JE 基因最初被描述为小鼠成纤维细胞中的血小板衍生生长因子诱导型基因（1）。研究发现，小鼠 JE 编码的蛋白质属于大型 CC 趋化因子家族，该家族包含炎症和免疫调节细胞因子。在 CC 趋化因子家族成员中，JE 在功能和结构上与 MCP/eotaxin 亚家族的蛋白质最为接近。在 MCP/eotaxin 亚家族中，已经鉴定出五种人蛋白（MCP-1、2、3、4 和 eotaxin）和四种小鼠蛋白（JE/MCP-1、MARC/MCP-3、MCP-5 和 eotaxin）（1-3）。在氨基酸序列水平上，成熟的人 MCP-1 与小鼠 JE、MARC 和 MCP-5 的相应区域分别显示出 55%、59%和 66%的同源性（1-3）。尽管 JE 一直被认为是人 MCP-1 的小鼠同源物（3-6），但最近分离出的小鼠 MCP-5 实际上更具同源性，可以被认为是第二个人 MCP-1 的同源物（7）。

小鼠 MCP-1 的 cDNA 编码一种由 148 个氨基酸（aa）残基组成的前体蛋白，预测有一个 23 aa 残基的信号肽，该信号肽经剪切后可生成一个由 125 aa 残基组成的成熟蛋白（1-3）。与成熟的人 MCP-1 相比，小鼠 MCP-1 具有一个由 49 aa 残基组成的羧基末端延伸段，该延伸段富含丝氨酸和苏氨酸残基。在 CHO 细胞中表达的重组 MCP-1（2）以及从小鼠星形胶质细胞（4,10）和小鼠胸腺上皮细胞系（8）中纯化得到的天然 MCP-1，均为约 30kDa 的糖蛋白，具有多个 O-寡糖链连接到 49 aa 残基的 C 末端结构域。然而，由病毒刺激的小鼠 L929 成纤维细胞产生的天然 MCP-1 却是一种非糖基化的 7-8kDa 蛋白，缺乏 C 末端结构域（12）。研究发现，C 末端结构域对于小鼠 MCP-1 的活性并非必需。除成纤维细胞、星形胶质细胞和上皮细胞外，小鼠 MCP-1 还在巨噬细胞（4, 9）、肥大细胞（7）、内皮细胞（7）、成骨细胞和成髓细胞中表达（11）。小鼠 MCP-1 在受到包括病毒、LPS 和细胞因子如 TNF-α、IL-1、IFN-γ和 PDGF 等炎症刺激后被诱导表达（1,3,4,12,13）。

小鼠 MCP-1 对单核细胞/巨噬细胞及淋巴细胞具有强大的趋化作用（3, 4, 13, 17）。研究还表明，它参与 Th1/Th2 淋巴细胞分化的调节，通过增加 IL-4 的产生和抑制 IL-12 的产生来促进 Th2 的发育（18-21）。小鼠 MCP-1 的活性已被证明由小鼠 CCR2 介导，CCR2 是一种 G 蛋白偶联的、具有七个跨膜结构域的受体（6, 14, 15）。小鼠 CCR2 的 cDNA 编码一个 373 aa 残基的蛋白质，该蛋白质在氨基酸序列水平上与人 MCP-1 受体 CCR2B 显示出最高的（80%）同源性（14-16）。小鼠 CCR2 基因已被定位到小鼠 9 号染色体上，与小鼠 CCR1 和 CCR3 基因紧密相邻。在单核细胞/巨噬细胞中检测到高水平的小鼠 CCR2 表达。

# II. 概述

## A. 检测原理

本实验采用双抗体夹心ELISA法。抗小鼠MCP-1抗体包被于微孔板上。样品，质控品和标准品中的小鼠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小鼠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，组织培养上清样本、组织裂解物、小鼠血浆样本和小鼠血清样本。
- 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RD5-3稀释后重新检测；
- 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。

| | 板内精确度 | | | 板间精确度 | | |
|---|---|---|---|---|---|---|
| 样本 | 1 | 2 | 3 | 1 | 2 | 3 |
| 平均值 (pg/mL) | 36.7 | 51.8 | 149 | 37.8 | 60.0 | 155 |
| 标准差 | 1.45 | 1.40 | 3.66 | 2.85 | 4.37 | 7.86 |
| CV% | 4.0 | 2.7 | 2.5 | 7.5 | 7.3 | 5.1 |

### B. 回收率

在不同类型样本中掺入检测范围内不同水平的小鼠 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= 4） | 105 | 95-112 |
| 裂解缓冲液（n= 4） | 88 | 79-101 |
| 小鼠血清（n=4） | 91 | 83-100 |
| 小鼠 EDTA 血浆（n=4） | 84 | 75-93 |
| 小鼠肝素血浆（n=4） | 93 | 80-101 |

### C. 灵敏度

22 次试验结果表明，小鼠 MCP-1 的最低检测剂量（MDD）的范围为 0.151-0.666 pg/mL 。平均 MDD 为 0.304 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小鼠 MCP-1 校正。

## E. 线性

不同的样本中含有或掺入高浓度的小鼠 MCP-1，然后用标准稀释液 RD5-3 稀释将样本稀释到检测范围内，测定其线性。

| | | 细胞培养上清 (n=4) | 裂解缓冲液 (n=4) | 小鼠血清 (n=4) | 小鼠 EDTA 血浆 (n=4) | 小鼠肝素血浆 * (n=4) |
|---|---|---|---|---|---|---|
| 1:2 | 平均值/期待值（%） | 105 | 99 | 96 | 96 | 107 |
| | 范围（%） | 102-107 | 95-108 | 94-99 | 93-104 | 104-109 |
| 1:4 | 平均值/期待值（%） | 110 | 102 | 101 | 102 | 111 |
| | 范围（%） | 106-116 | 94-107 | 97-109 | 101-105 | 104-116 |
| 1:8 | 平均值/期待值（%） | 109 | 109 | 105 | 107 | 104 |
| | 范围（%） | 105-113 | 103-116 | 100-116 | 100-110 | 98-110 |
| 1:16 | 平均值/期待值（%） | 115 | 119 | 106 | 107 | 108 |
| | 范围（%） | 107-125 | 113-124 | 101-116 | 104-112 | 102-119 |

*样品在测定前按照样品制备部分的指示进行稀释。

## F. 样本预值

**小鼠血清/血浆样本 -** 在此测定中对样品进行小鼠 MCP-1 的可检测水平的评估。

| 小鼠样本 | 平均值(pg/mL) | 范围 (pg/mL) | 标准偏差 (pg/mL) |
|---|---|---|---|
| 小鼠血清 (n=10) | 114 | 64.5-216 | 44.7 |
| 小鼠 EDTA 血浆 (n=10) | 76.6 | 42.5-116 | 23.8 |
| 小鼠肝素血浆 (n=10) | 96.6 | 72.2-154 | 25.9 |

**细胞培养上清** - C2C12 小鼠成肌细胞培养在含10% 胎牛血清的 DMEM 培养基中，培养至汇合。用PBS冲洗细胞，并让细胞培养在含5%马血清的DMEM培养基中，培养5-7天，每隔一天更换一次培养基。当细胞分化到50 %时，不刺激或用脂多糖（LPS）刺激细胞，培养3天。取出等量的细胞培养上清，检测小鼠MCP-1的水平，检测值分别为 2359 pg/mL和11993 pg/mL。

J774A.1小鼠网状细胞肉瘤巨噬细胞，培养在含10% 胎牛血清和 2 mM L-谷氨酰胺的RPMI培养基中。不刺激或用脂多糖（LPS）刺激细胞，培养3天。取出等量的细胞培养上清，检测小鼠MCP-1的水平，检测值分别为 8430 pg/mL和34717 pg/mL。

**组织培养上清** - 将小鼠脾脏细胞匀浆，培养在含10%胎牛血清、2 mM L-谷氨酰胺、100 U/mL青霉素和100 µg/mL硫酸链霉素的 RPMI 1640培养基中，培养2天。取出等量的细胞培养上清，检测小鼠MCP-1的水平，检测值为24.7 pg/mL。

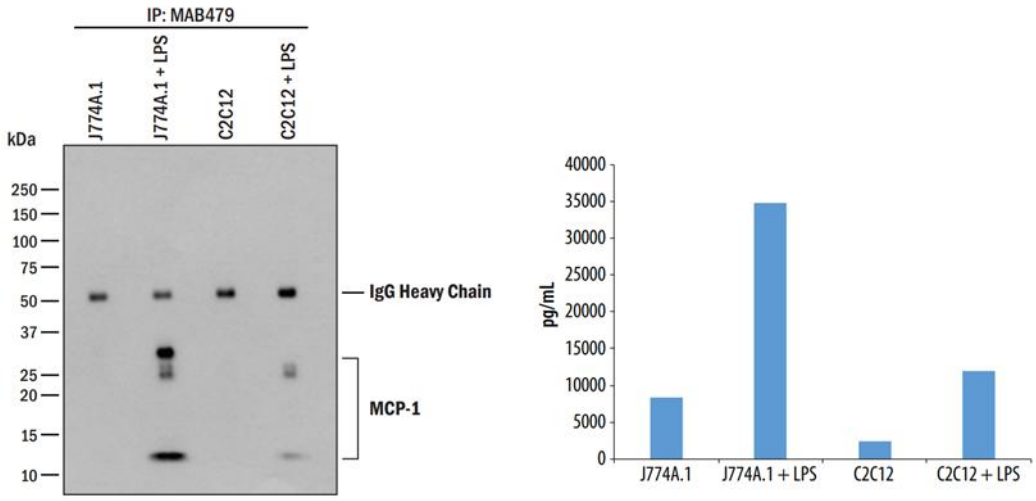
**组织裂解物** - 用PBS在冰上冲洗小鼠肺组织。倒掉PBS，加入10 mL含蛋白酶抑制剂的冰冷裂解缓冲液。用匀浆器在冰上匀浆 2 × 5 秒，使组织均质化。样品在2000 rpm离心 5 分钟。建议使用总蛋白测定法对样本蛋白质浓度进行定量。取50 µg组织裂解物检测小鼠MCP-1，检测值为26.8 pg/mL。

## G. 特异性

此ELISA法可检测天然及重组小鼠MCP-1。

将以下表用标准品稀释液RD5-3配制成50 ng/mL的浓度来检测与小鼠MCP-1的交叉反应。将50 ng/mL的干扰因子掺入中间范围的重组小鼠MCP-1对照品中，来检测对小鼠/MCP-1的干扰。未观察到明显的交叉反应或干扰

| **Recombinant mouse:** | | **Other recombinant:** |
|---|---|---|
| Eotaxin | MIP-1α | human MCP-1 |
| MARC | MIP-1β | rat MCP-1 |
| MCP-2 | MIP-1γ | |
| MCP-5 | MIP-3α | |

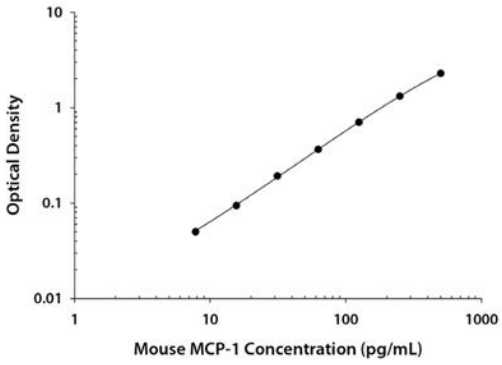



通过免疫沉淀 Western Blot 和 Valukine ELISA 分析未经处理或经 LPS 处理 72 小时的 J774A.1 和 C2C12 细胞的条件培养基。使用大鼠抗小鼠 CCL2/JE/MCP-1（R&D Systems®，目录编号 MAB479）对条件培养基进行免疫沉淀。对于 Western Blot，样品在还原 SDS-PAGE 条件下电泳，转移到 PVDF 膜上，并用山羊抗小鼠 CCL2/JE/MCP-1（R&D Systems®，产品目录 # AF-479-NA）进行免疫印迹。免疫沉淀 Western Blot 显示这些样品与 ELISA 值直接相关。

# 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。




| (pg/mL) | O.D. | Average | Corrected |
|---|---|---|---|
| 0 | 0.007<br>0.007 | 0.007 | — |
| 7.81 | 0.056<br>0.057 | 0.057 | 0.050 |
| 15.6 | 0.100<br>0.101 | 0.101 | 0.094 |
| 31.3 | 0.196<br>0.202 | 0.199 | 0.192 |
| 62.5 | 0.365<br>0.379 | 0.372 | 0.365 |
| 125 | 0.702<br>0.715 | 0.709 | 0.702 |
| 250 | 1.320<br>1.332 | 1.326 | 1.319 |
| 500 | 2.274<br>2.317 | 2.296 | 2.289 |

## V. 试剂盒组成及储存

### A. 试剂盒组成

| 组成 | 描述 | 规格 |
|---|---|---|
| Mouse MCP-1 Microplate | 包被抗小鼠 MCP-1 抗体的 96 孔聚苯乙烯板，8 孔× 12 条 | 1 块板 |
| Mouse MCP-1 Conjugate | 酶标检测抗小鼠 MCP-1 抗体 | 1 瓶 |
| Mouse MCP-1 Standard | 小鼠 MCP-1 标准品（冻干），参考瓶身标签进行重溶 | 2 瓶 |
| Mouse MCP-1 Control | 小鼠 MCP-1 质控品（冻干），质控品的测定值应在标签上规定的范围内 | 2 瓶 |
| Assay Diluent RD1W | 检测液 | 1 瓶 |
| Calibrator Diluent RD5-3 | 标准品稀释液用于稀释标准品和样本 | 1 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="5">2-8°C 储存，最多 30 天*</td></tr>
<tr><td>检测液 RD1W</td></tr>
<tr><td>标准品稀释液<br>RD5-3</td></tr>
<tr><td>终止液</td></tr>
<tr><td>酶标检测抗体</td></tr>
<tr><td>TMB 底物溶液</td><td rowspan="2">每次检测均使用新的标准及对照，用后丢弃。</td></tr>
<tr><td>质控品</td></tr>
<tr><td>标准品</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；<br>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
- 500 mL 量筒
- 用于稀释标准品和样品的管子

## D. 组织裂解物样本所需的用品

- 细胞裂解缓冲液 2 (R&D Systems，货号# 895347)，细胞裂解缓冲液 3 (R&D Systems，货号# 895366)，裂解缓冲液 16 (R&D Systems，货号# 895935)，或裂解缓冲液 17 (R&D Systems，货号# 895943)
- PBS

## E. 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：**颗粒物应通过离心去除；立即检测样本或分装，≤ -20 °C储存备用，避免反复冻融。样本可能需要用标准品稀释液RD5-3稀释。

**组织培养上清液：** 在检测前必须按照样本值部分的说明制备组织。样品可能需要用标准品稀释液RD5-3稀释。

**组织裂解物：**按照样本值部分所述在检测前制备裂解物。样品可能需要用标准品稀释液RD5-3稀释。

**血清样本：**血液样品在室温下凝集2小时，然后在2000 × g下离心20分钟。吸取血清样本之后即刻用于检测，或者分装，≤ -20°C储存备用。避免反复冻融。样本可能需要用标准品稀释液 RD5-3 稀释。

**血浆样本：**使用EDTA或肝素作为抗凝剂收集血浆。然后2000 × g离心20分钟。需在30分钟内收集血浆样本之后即刻用于检测，或者分装，≤ -20°C储存备用。避免反复冻融。样本可能需要用标准品稀释液RD5-3稀释。

***注意：****本试剂盒对柠檬酸钠血浆尚未被验证。*

## B. 检测前准备工作

**使用前请将所有试剂放置于室温。**

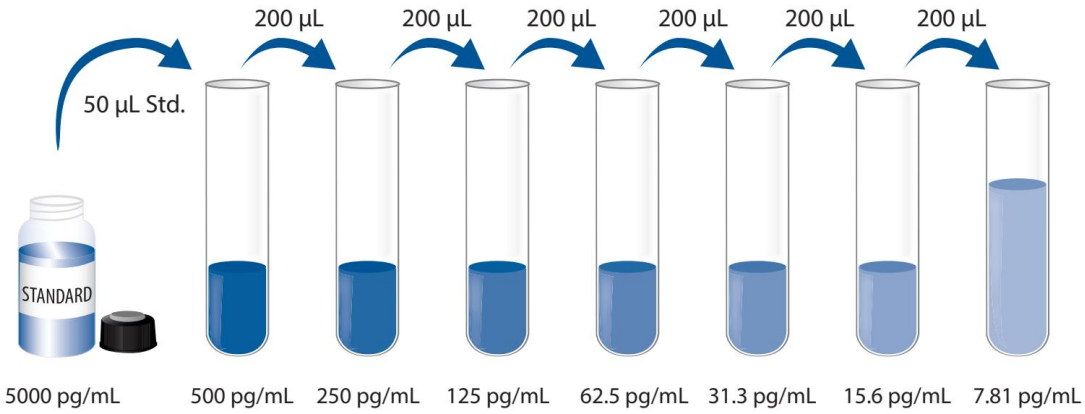
**小鼠MCP-1质控品：**使用1.0 mL去离子水或蒸馏水重溶质控品。混合均匀，测定时不稀释质控品。

**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×）。

**小鼠MCP-1标准品：**重溶体积请参考瓶身标签*，用去离子水或蒸馏水重溶小鼠 MCP-1 标准品。不得使用其他试剂。得到浓度为5000 pg/mL标准品母液。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。

**加入450 µL标准品稀释液RD5-3到 500 pg/mL 管中。剩余每管中加入200 µL标准品稀释液RD5-3**。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。500 pg/mL作标准曲线最高点，**标准品稀释液RD5-3**可用作标准曲线零点（0 pg/mL）。




## C. 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

**使用前请将所有试剂和样本放置于室温，建议所有的实验样本，质控品和标准品做复孔检测。**

1. 按照上一节的说明，准备好所有需要的试剂，标准品，质控品和样本；
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. 每孔加入50μL检测液RD1W。
4. 分别将不同浓度标准品，质控品和实验样本加入相应孔中，每孔50 μL。用封板膜封住反应孔，**室温孵育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3次，共洗4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100 μL小鼠MCP-1酶标检测抗体。用封板膜封住反应孔，**室温孵育2小时；**
7. 重复第5步洗板操作
8. 在每个微孔内加入100 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100 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，质控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。数据可以通过绘制小鼠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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## 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