



## **PRODUCT INFORMATION & MANUAL**

**Human CCL4/MIP-1 $\beta$  Valukine™ ELISA**

**VAL161**

For the quantitative determination of natural and recombinant  
human CCL4/MIP-1 $\beta$  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 202206.1

# TABLE OF CONTENTS

I. BACKGROUND .....	2
II. OVERVIEW .....	3
III. ADVANTAGES .....	4
IV. EXPERIMENT .....	6
V. KIT COMPONENTS AND STORAGE .....	7
VI. PREPARATION .....	9
VII. ASSAY PROCEDURE .....	12
VIII. REFERENCES .....	14

## I. BACKGROUND

CCL4, also known as macrophage inflammatory protein 1 beta (MIP-1 beta) is a 7.8 kDa beta chemokine that is secreted at sites of inflammation by activated leukocytes, lymphocytes, vascular endothelial cells, and pulmonary smooth muscle cells (1, 2). CCL4 attracts a variety of immune cells to sites of microbial infection as well as to other pathologic inflammation such as allergic asthma and ischemic myocardium (3-8). A CCL4 deficiency in mice promotes the development of autoantibodies, possibly as a result of compromised regulatory T cell recruitment (6). CCL4 is secreted from activated monocytes as a heterodimer with CCL3/MIP-1 alpha (9). The first two N-terminal amino acids can be cleaved from human CCL4 by CD26/DPPIV (10, 11). Both the full length and truncated forms exert biological activity through CCR5, and the truncated form additionally interacts with CCR1 and CCR2b (10). In humans, the ability of CCL4 to bind CCR5 inhibits the cellular entry of M-tropic HIV-1 which utilizes CCR5 as a coreceptor (2). Both forms of CCL4 block HIV-1 infection of T cells by inducing the downregulation of CCR5 (10). Mature human CCL4 shares 77% and 80% aa sequence identity with mouse and rat CCL4, respectively.

## II. OVERVIEW

### A. PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. An antibody specific for human CCL4/MIP-1 $\beta$  has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any CCL4/MIP-1 $\beta$  present is bound by the immobilized antibody. After washing away any unbound substances, a biotinylated detection antibody specific for human CCL4/MIP-1 $\beta$  is pipetted into the wells. After washing away any unbound substances, streptavidin-HRP is pipetted into the wells. Following a wash to remove any unbound reagent, a substrate solution is added to the wells and color develops in proportion to the amount of CCL4/MIP-1 $\beta$  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 and human 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 Reagent Diluent or Calibrator Diluent 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)

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

**Inter-assay Precision** (Precision between assays)

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

	Intra-assay Precision				Inter-assay Precision			
Sample	1	2	3	4	1	2	3	4
Mean (pg/mL)	1950.1	545.2	153.2	70.1	2060.8	535.4	146.1	69.9
Standard Deviation	179.1	25.5	13.7	2.2	188.9	23.2	13.2	1.9
CV%	9.2	4.7	8.9	3.2	9.2	4.3	9.1	2.7

#### B. RECOVERY

The recovery of human CCL4/MIP-1 $\beta$  spiked to different levels throughout the range of the assay in cell culture media was evaluated. The recovery ranged from 70.7-92.5% with an average of 81.1%.

The recovery of human CCL4/MIP-1 $\beta$  spiked to different levels throughout the range of the assay in human serum was evaluated. The recovery ranged from 82.5-121.6% with an average of 101.4%.

#### C. SENSITIVITY

The minimum detectable dose (MDD) of human CCL4/MIP-1 $\beta$  is typically less than 6.02 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 CCL4/MIP-1 $\beta$  produced at R&D Systems.

## E. LINEARITY

To assess the linearity of the assay, different samples were containing or spiked with high concentrations of human CCL4/MIP-1 $\beta$  and diluted with Reagent Diluent 1 $\times$  (*for cell culture supernate samples*) or Calibrator Diluent 1 $\times$  (*for serum samples*) to produce samples with values within the dynamic range of the assay.

Dilution	Average % of Expected	Range (%)
1:2	98.6	77.2-115.8
1:4	98.6	80.5-118.5
1:8	101.9	77.5-124.5
1:16	104.0	86.6-113.5

## F. SAMPLE VALUES

**Serum** - Seven human serum samples were evaluated for the presence of human CCL4/MIP-1 $\beta$  in this assay. All samples measured ranged from 188.0 to 490.8 pg/mL with an average of 382.7 pg/mL.

## G. SPECIFICITY

The following factors prepared at 50 ng/mL were assayed and exhibited no cross-reactivity or interference.

Recombinant human	Recombinant Mouse
GRO $\alpha$	MIP-1 $\alpha$
GRO $\beta$	MIP-1 $\beta$
GRO $\gamma$	MIP-1 $\gamma$
MCP-1	MIP-3 $\alpha$
MIP-1 $\alpha$	MIP-3 $\beta$
MIP-1 $\delta$	<b>Other recombinants</b>
MIP-3 $\alpha$	rat MIP-3 $\alpha$
MIP-3 $\beta$	viral MIP-I
RANTES	viral MIP-II

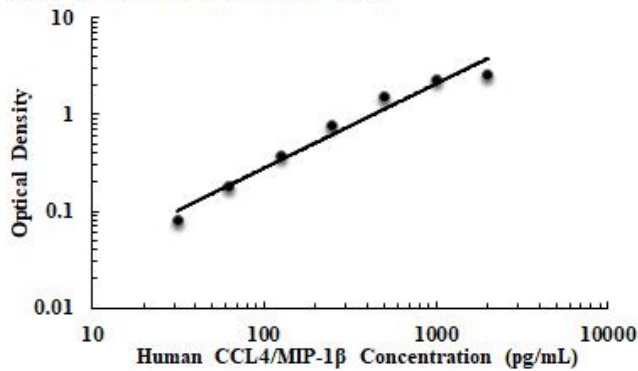
A sample containing 3.13 ng/mL of recombinant human MIP-1 $\alpha$  (70 aa) reads as 56.6 pg/mL (1.81% cross-reactivity).

## IV. EXPERIMENT

### EXAMPLE STANDARD

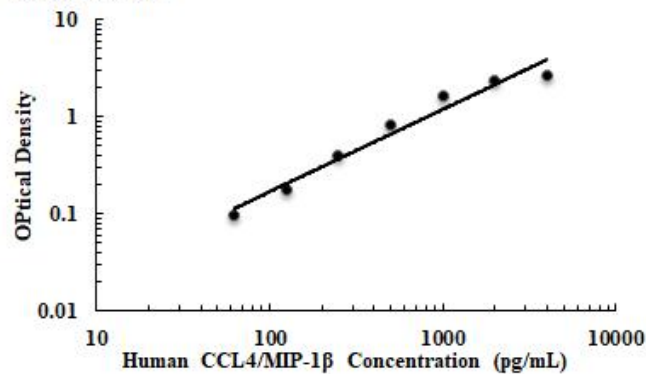
The standard curve is provided for demonstration only. A standard curve should be generated for each set of samples assayed.

CELL CULTURE SUPERNATE ASSAY



pg/mL	OD	Average	Corrected
0	0.089 0.090	0.090	-
31.3	0.169 0.172	0.171	0.081
62.5	0.263 0.272	0.268	0.178
125	0.453 0.459	0.456	0.367
250	0.863 0.863	0.863	0.774
500	1.566 1.602	1.584	1.495
1000	2.361 2.366	2.364	2.274
2000	2.684 2.702	2.693	2.604

SERUM ASSAY



pg/mL	OD	Average	Corrected
0	0.074 0.075	0.075	-
62.5	0.173 0.173	0.173	0.099
125	0.243 0.259	0.251	0.177
250	0.456 0.477	0.467	0.392
500	0.898 0.930	0.914	0.840
1000	1.680 1.686	1.683	1.609
2000	2.367 2.450	2.409	2.334
4000	2.698 2.729	2.714	2.639

## V. KIT COMPONENTS AND STORAGE

### A. MATERIALS PROVIDED

Parts	Description	Size
Human CCL4/MIP-1 $\beta$ Microplate	96 well polystyrene microplate (12 strips of 8 wells) coated with a mouse antibody against human CCL4/MIP-1 $\beta$ .	1 plate
Human CCL4/MIP-1 $\beta$ Standard	Recombinant human CCL4/MIP-1 $\beta$ in a buffered protein base; lyophilized. Refer to the vial label for reconstitution volume.	2 vials
Human CCL4/MIP-1 $\beta$ Detection Antibody	Biotinylated CCL4/MIP-1 $\beta$ antibody, lyophilized. Refer to the vial label for reconstitution volume.	2 vials
Streptavidin-HRP B (40 $\times$ )	40 $\times$ Streptavidin conjugated to horseradish peroxidase.	1 vial
Reagent Diluent (10 $\times$ )	A 10 $\times$ concentrated buffered base used to dilute standard, samples, Detection Antibody and HRP.	1 vial
Calibrator Diluent (1 $\times$ )	buffer diluent used to dilute standard and samples.	2 vials
Detection Antibody Diluent (4 $\times$ )	A 4 $\times$ concentrated buffered protein base used to dilute Detection Antibody.	1 vial
Wash Buffer Concentrate (25 $\times$ )	A 25 $\times$ concentrated solution of buffered surfactant with preservatives.	1 vial
Color Reagent A	Stabilized hydrogen peroxide.	1 vial
Color Reagent B	Stabilized chromogen (tetramethylbenzidine).	1 vial
Stop Solution	2 N sulfuric acid.	1 vial
Plate Sealers	Adhesive strip.	3 strips

### B. STORAGE

Unopened Kit	Store at 2-8 °C. Do not use past kit expiration date.	
Opened/ Reconstituted Reagents	Streptavidin-HRP B	May be stored for up to 1 month at 2-8 °C.*
	Diluted Wash Solution	
	Unmixed Color Reagent A	



	Unmixed Color Reagent B	
	Stop Solution	
	Standard	Prepare fresh for each assay.
	Detection Antibody	Aliquot and store for up to 1 month at -20 °C in a manual defrost freezer. *
	Reagent Diluent (10×)	May be stored for up to 1 month at 2-8 °C.* Use and discard diluted Reagent Diluent (1×). Prepare fresh for each assay.
	Calibrator Diluent (1×)	May be stored for up to 1 month at 2-8 °C.*
	Detection Antibody Diluent (4×)	May be stored for up to 1 month at 2-8 °C.* Use and discard diluted Detection Antibody Diluent (1×). Prepare fresh for each assay.
	Microplate Wells	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.*

\* 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.
- ◆ An incubator which can provide stable incubation conditions up to 25°C ± 0.5°C.

### D. PRECAUTION

- ◆ 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.
- ◆ The Stop Solution provided with this kit is an acid solution. Wear protective gloves, clothing, eye, and face protection. Wash hands thoroughly after handling.

## VI. PREPARATION

### A. SAMPLE COLLECTION AND STORAGE

**Cell Culture Supernates** - Remove particulates by centrifugation and assay immediately or aliquot and store samples at  $\leq -20^{\circ}\text{C}$ . Avoid repeated freeze-thaw cycles. Samples may require dilution with Reagent Diluent (1 $\times$ ).

**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  $\leq -20^{\circ}\text{C}$ . Avoid repeated freeze-thaw cycles. Samples may require dilution with Calibrator Diluent (1 $\times$ ).

### B. SAMPLE PREPARATION

Cell culture supernate samples require a 2-fold dilution prior to the assay. A suggested 2-fold dilution is 100  $\mu\text{L}$  of sample + 100  $\mu\text{L}$  of **Reagent Diluent (1 $\times$ )**.

Human serum samples require a 2-fold dilution. A suggested 2-fold dilution is 100  $\mu\text{L}$  of sample + 100  $\mu\text{L}$  of **Calibrator Diluent (1 $\times$ )**.

### C. REAGENT PREPARATION

*Note: 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. Dilute 20 mL of Wash Buffer Concentrate (25 $\times$ ) into deionized or distilled water to prepare 500 mL of Wash Buffer.

**Reagent Diluent (1 $\times$ )** - Use deionized or distilled water to prepare Reagent Diluent (1 $\times$ ).

**Detection Antibody Diluent (1 $\times$ )** - Use deionized or distilled water to prepare Detection Antibody Diluent (1 $\times$ ).

**Detection Antibody- Reconstitution Volume refer to vial label with Reagent Diluent (1 $\times$ )**. Aliquot and store if needed. Dilute stock solution in Detection Antibody Diluent (1 $\times$ ) to the working concentration of 50 ng/mL. Prepare at least 15 minutes prior to use.

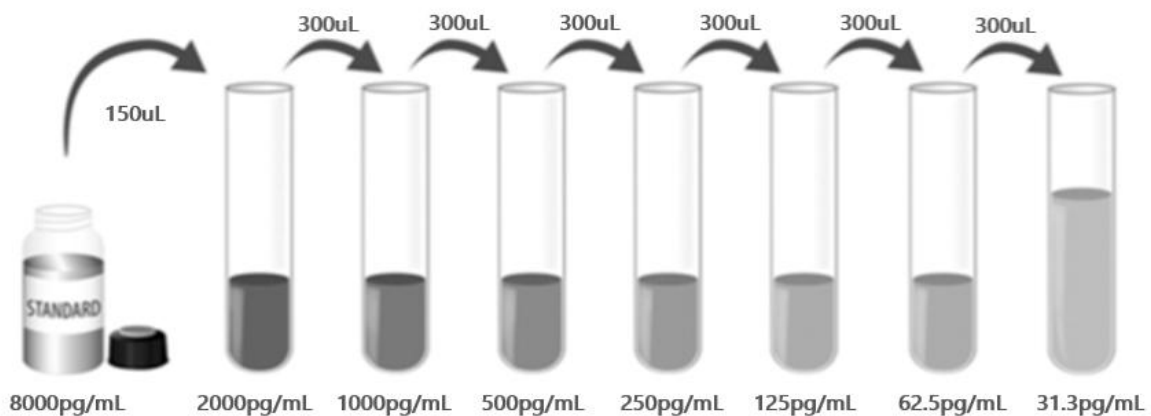
**Streptavidin-HRP B (1 $\times$ )** - **Centrifuge briefly before opening**. Dilute to the working concentration specified on the vial label using Reagent Diluent (1 $\times$ ).

**Substrate Solution** - Color Reagent A and B should be mixed together in equal volumes within 15 minutes of use. Protect from light. 100  $\mu$ L of the resultant mixture is required per well.

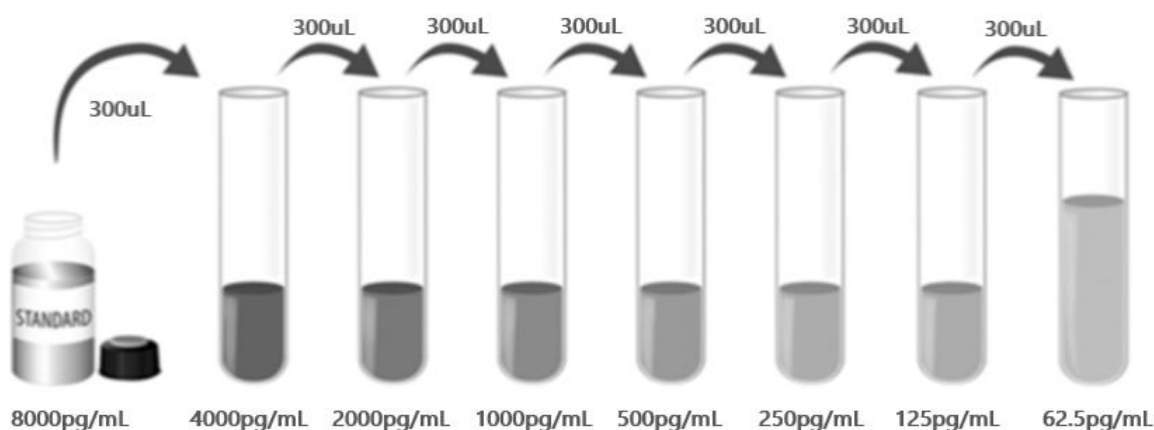
**Human CCL4/MIP-1 $\beta$  Standard-** Refer to the vial label for the reconstitution volume\*. Reconstitute the human CCL4/MIP-1 $\beta$  Standard with **Reagent Diluent (1 $\times$ )** (for cell culture supernate samples) or **Calibrator Diluent (1 $\times$ )** (for serum samples). This reconstitution produces a stock solution of 8000 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.

**For Cell Supernate Samples-** Pipette 450  $\mu$ L of **Reagent Diluent (1 $\times$ )** (for cell culture supernate samples) into the 2000 pg/mL tube. Pipette 300  $\mu$ L of **Reagent Diluent (1 $\times$ )** into the remaining tubes. Use the stock solution to produce a dilution series (below). Mix each tube thoroughly before the next transfer. The 2000 pg/mL standard serves as the high standard. The **Reagent Diluent (1 $\times$ )** serves as the zero standard (0 pg/mL).



**For Serum Samples-** Pipette 300  $\mu$ L of **Calibrator Diluent (1 $\times$ )** (for serum samples) into each tube. Use the stock solution to produce a dilution series (below). Mix each tube thoroughly before the next transfer. The 4000 pg/mL standard serves as the high standard. The **Calibrator Diluent (1 $\times$ )** 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.
- 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.

## VII. ASSAY PROCEDURE

**Note:** Bring all reagents and samples to room temperature before use. It is recommended that all samples and standards be assayed in duplicate.

1. Prepare all reagents and working standards 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 100  $\mu$ L of standard, or prepared sample per well. Cover with the adhesive strip provided. Incubate for 2 hours **in an incubator at 25°C**. A plate layout is provided for a record of standards and samples assayed. (Samples may require dilution. See Sample Preparation section.)
4. Aspirate each well and wash, repeating the process three times for a total of four washes. Wash by filling each well with Wash Solution (400  $\mu$ 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.
5. Add 100  $\mu$ L of the Detection Antibody diluted in Detection Antibody Diluent to each well. Cover with a new adhesive strip and incubate 2 hours **in an incubator at 25°C**.
6. Repeat the aspiration/wash as in step 4.
7. Add 100  $\mu$ L of the working dilution of Streptavidin-HRP B to each well. Cover the plate and incubate for 30 minutes **in an incubator at 25°C**. Avoid placing the plate in direct light.
8. Repeat the aspiration/wash as in step 4.
9. Add 100  $\mu$ L of Substrate Solution to each well. Incubate for 30 minutes **in an incubator at 25°C**. Avoid placing the plate in direct light.
10. Add 50  $\mu$ L of Stop Solution to each well. Gently tap the plate to ensure thorough mixing.
11. Determine the optical density of each well immediately, 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.

## 12. **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 CCL4/MIP-1 $\beta$  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.

<b>1</b>								
<b>2</b>								
<b>3</b>								
<b>4</b>								
<b>5</b>								
<b>6</b>								
<b>7</b>								
<b>8</b>								
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<b>10</b>								
<b>11</b>								
<b>12</b>								
	<b>A</b>	<b>B</b>	<b>C</b>	<b>D</b>	<b>E</b>	<b>F</b>	<b>G</b>	<b>H</b>





## 产品信息及操作手册

人 CCL4/MIP-1 $\beta$  Valukine™ ELISA 试剂盒

目录号: VAL161

适用于定量检测天然和重组人 CCL4/MIP-1 $\beta$ 的浓度

科研专用, 不可用于临床诊断

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## 目录

I. 背景 .....	18
II. 概述 .....	19
III. 优势 .....	20
IV. 实验 .....	22
V. 试剂盒组成及储存 .....	23
VI. 实验前准备 .....	25
VII. 操作步骤 .....	27
VIII. 参考文献 .....	28

## I. 背景

CCL4，也被称为巨噬细胞炎症蛋白1 $\beta$ （MIP-1 $\beta$ ），是一种7.8 kDa的 $\beta$ 趋化因子，在炎症部位由活化的白细胞、淋巴细胞、血管内皮细胞和肺平滑肌细胞分泌（1, 2）。CCL4将多种免疫细胞吸引到微生物感染部位，以及其他病理性炎症部位，如过敏性哮喘和缺血性心肌（3-8）。小鼠CCL4缺乏会促进自身抗体的产生，这可能是调节性T细胞募集受损的结果（6）。CCL4是由活化的单核细胞分泌，与CCL3/MIP-1 $\alpha$ 形成异质二聚体（9）。人CCL4的前两个N端氨基酸可以被CD26/DPPIV切割（10, 11）。其全长和截短形式都是通过CCR5发挥生物活性，截短的形式还与CCR1和CCR2b相互作用（10）。在人类中，CCL4结合CCR5的能力可以抑制M-tropic HIV-1进入细胞，而M-tropic HIV-1利用CCR5作为一种共受体（2）。两种形式的CCL4通过诱导CCR5的下调来阻断HIV-1对T细胞的感染（10）。成熟的人CCL4与小鼠和大鼠CCL4的氨基酸序列同源性分别为77%和80%。

## II. 概述

### A. 检测原理

本实验采用双抗体夹心ELISA法。小鼠抗人CCL4/MIP-1 $\beta$ 抗体包被于微孔板上，样品和标准品中的人CCL4/MIP-1 $\beta$ 会与固定在板上的抗体结合，游离的成分被洗去；接着加入生物素化的抗人CCL4/MIP-1 $\beta$ 检测抗体进行孵育，洗涤去除未结合的物质后，加入链霉亲和素标记的辣根过氧化物酶（streptavidin-HRP）孵育。洗涤去除未结合的试剂后，加入底物溶液（显色剂）。溶液颜色与结合的目标蛋白成正比；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和人血清样本；
- ◆ 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，应将样本用试剂稀释液（1 $\times$ ）或标准品稀释液（1 $\times$ ）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、试剂盒的储存等。

### III. 优势

#### A. 精确度

**板内精确度**（同一板内不同孔间的精确度）

已知浓度的四个样本，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）

已知浓度的四个样本，在不同板中分别检测20次，以确定板间精确度。

样本	板内精确度				板间精确度			
	1	2	3	4	1	2	3	4
平均值 (pg/mL)	1950.1	545.2	153.2	70.1	2060.8	535.4	146.1	69.9
标准差	179.1	25.5	13.7	2.2	188.9	23.2	13.2	1.9
CV%	9.2	4.7	8.9	3.2	9.2	4.3	9.1	2.7

#### B. 回收率

在细胞培养基样本中掺入检测范围内不同水平的人CCL4/MIP-1 $\beta$ ，测定其回收率。回收率范围在70.7-92.5%，平均回收率在81.1%。

在人血清样本中掺入检测范围内不同水平的人CCL4/MIP-1 $\beta$ ，测定其回收率。回收率范围在82.5-121.6%，平均回收率在101.4%。

#### C. 灵敏度

人CCL4/MIP-1 $\beta$ 的最低可测量（MDD）一般小于6.02 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

此ELISA试剂盒经由R&D Systems生产的大肠杆菌表达的高纯度重组人CCL4/MIP-1 $\beta$ 蛋白所校正。

#### E. 线性

不同的样本中含有或掺入高浓度的人CCL4/MIP-1 $\beta$ ，然后用试剂稀释液（1 $\times$ ）（用于细

胞上清样本) 或标准品稀释液 (1×) (用于人血清样本) 将样本稀释到检测范围内, 测定其线性。

稀释倍数	平均值 (%)	范围 (%)
1:2	98.6	77.2-115.8
1:4	98.6	80.5-118.5
1:8	101.9	77.5-124.5
1:16	104.0	86.6-113.5

#### F. 样本预值

**血清样本** - 使用本试剂盒检测了7份人血清样本中CCL4/MIP-1 $\beta$ 的水平。7份样本的检测值范围为188.0-490.8 pg/mL, 平均值为382.7 pg/mL。

#### G. 特异性

将以下因子配制成50 ng/mL的浓度来检测没有观察到明显的交叉反应或干扰。

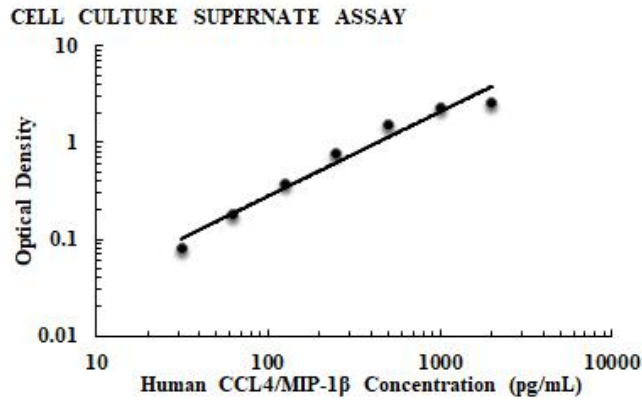
Recombinant human	Recombinant Mouse
GRO $\alpha$	MIP-1 $\alpha$
GRO $\beta$	MIP-1 $\beta$
GRO $\gamma$	MIP-1 $\gamma$
MCP-1	MIP-3 $\alpha$
MIP-1 $\alpha$	MIP-3 $\beta$
MIP-1 $\delta$	<b>Other recombinants</b>
MIP-3 $\alpha$	rat MIP-3 $\alpha$
MIP-3 $\beta$	viral MIP-I
RANTES	viral MIP-II

含有3.13 ng/mL重组人CCL4/MIP-1 $\alpha$ 的样品检测值为56.6 pg/mL (交叉反应率为1.81%)。

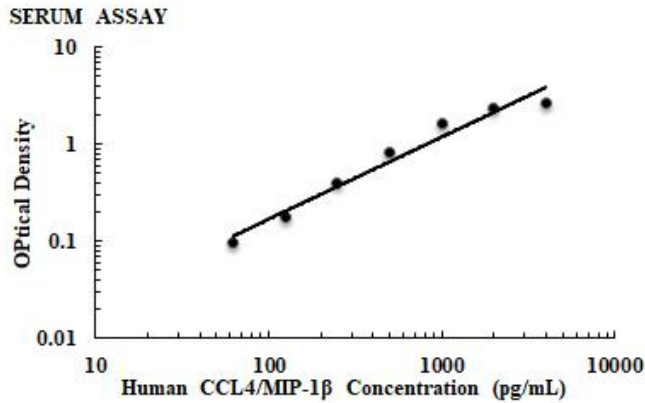
## IV. 实验

### 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	OD	Average	Corrected
0	0.089 0.090	0.090	-
31.3	0.169 0.172	0.171	0.081
62.5	0.263 0.272	0.268	0.178
125	0.453 0.459	0.456	0.367
250	0.863 0.863	0.863	0.774
500	1.566 1.602	1.584	1.495
1000	2.361 2.366	2.364	2.274
2000	2.684 2.702	2.693	2.604



pg/mL	OD	Average	Corrected
0	0.074 0.075	0.075	-
62.5	0.173 0.173	0.173	0.099
125	0.243 0.259	0.251	0.177
250	0.456 0.477	0.467	0.392
500	0.898 0.930	0.914	0.840
1000	1.680 1.686	1.683	1.609
2000	2.367 2.450	2.409	2.334
4000	2.698 2.729	2.714	2.639

## V. 试剂盒组成及储存

### A. 试剂盒组成

组成	描述	规格
Human CCL4/MIP-1 $\beta$ Microplate	包被小鼠抗人 CCL4/MIP-1 $\beta$ 抗体的 96 孔聚苯乙烯板, 8 孔 $\times$ 12 条	1 块板
Human CCL4/MIP-1 $\beta$ Standard	标准品 (冻干粉), 参考瓶身标签进行重溶	2 瓶
Human CCL4/MIP-1 $\beta$ Detection Antibody	生物素化的 CCL4/MIP-1 $\beta$ 检测抗体, 冻干粉, 参考瓶身标签进行重溶	2 瓶
Streptavidin-HRP B (40 $\times$ )	40 $\times$ 浓缩的链霉亲和素标记的 HRP	1 瓶
Reagent Diluent (10 $\times$ )	浓缩的试剂稀释液 (10 $\times$ )	1 瓶
Calibrator Diluent (1 $\times$ )	标准品稀释液 (1 $\times$ )	2 瓶
Detection Antibody Diluent (4 $\times$ )	浓缩的检测抗体稀释液 (4 $\times$ )	1 瓶
Wash Buffer Concentrate (25 $\times$ )	浓缩洗涤缓冲液 (25 $\times$ )	1 瓶
Color Reagent A	显色液 A	1 瓶
Color Reagent B	显色液 B	1 瓶
Stop Solution	终止液	1 瓶
Plate Sealers	封板膜	3 张

### B. 试剂盒储存

未开封试剂盒	2-8 $^{\circ}$ C 储存; 请在试剂盒有效期内使用	
已打开, 稀释或重溶的试剂	链霉亲和素-HRP B	2-8 $^{\circ}$ C 储存, 最多 30 天*
	洗涤缓冲液 (1 $\times$ )	
	显色液 A	
	显色液 B	



	终止液	
	标准品	使用时新鲜配制*
	检测抗体	分装， -20℃ 储存，最多 30 天*
	标准品稀释液（1×）	2-8℃ 储存，最多 30 天*
	试剂稀释液（10×）	2-8℃ 储存，最多 30 天* 请每次使用新鲜配制的 1× 试剂稀释液
	检测抗体稀释液（4×）	2-8℃ 储存，最多 30 天* 请每次使用新鲜配制的 1× 检测抗体稀释液
	包被的微孔板条	将未用的板条放回带有干燥剂的铝箔袋内， 密封：2-8℃ 储存，最多 30 天*

\*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
- ◆ 25℃恒温箱

### 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。
- ◆ 显色液B可能引起皮肤、眼睛和呼吸道刺激，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、面部及衣服的保护。使用后请彻底洗手。

## VI. 实验前准备

### A. 样品收集及储存

**细胞培养上清液:** 颗粒物应离心去除; 立刻检测样本。样本收集后若不及时检测, 需按一次使用量分装, 冻存于-20℃冰箱内, 避免反复冻融。样本可能需要用试剂稀释液 (1×) 稀释。

**血清样本:** 用血清分离管(SST)分离血清。使血样室温凝集30分钟, 然后1000 x g离心15分钟。吸取血清样本之后即刻用于检测, 或者分装, -20℃贮存备用。避免反复冻融。样本可能需要用标准品稀释液 (1×) 稀释。

### B. 样本准备工作

细胞上清样本需要用**试剂稀释液 (1×)** 2倍稀释后进行检测, 例如: 100 μL细胞上清液+100 μL试剂稀释液 (1×)。

血清样本需要用**标准品稀释液 (1×)** 2倍稀释后进行检测, 例如: 100 μL血清样本+100 μL标准品稀释液 (1×)。

### 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:** 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20mL浓缩洗涤液用去离子水或蒸馏水稀释配制成500mL工作浓度的洗涤液。

**试剂稀释液 (1×):** 使用去离子水或蒸馏水稀释配制成试剂稀释液 (1×)。

**检测抗体稀释液 (1×):** 使用去离子水或蒸馏水稀释配制成检测抗体稀释液 (1×)。

**检测抗体:** 参考检测抗体瓶标签指示, 用试剂稀释液 (1×) 将冻干粉进行重溶。再用检测抗体稀释液 (1×) 稀释至工作浓度50ng/mL, 至少在使用前15分钟准备。

**链霉亲和素- HRP B:** 开盖前请瞬时离心。用试剂稀释液 (1×) 将链霉亲和素- HRP B (40×) 稀释至工作浓度链霉亲和素- HRP B (1×)。

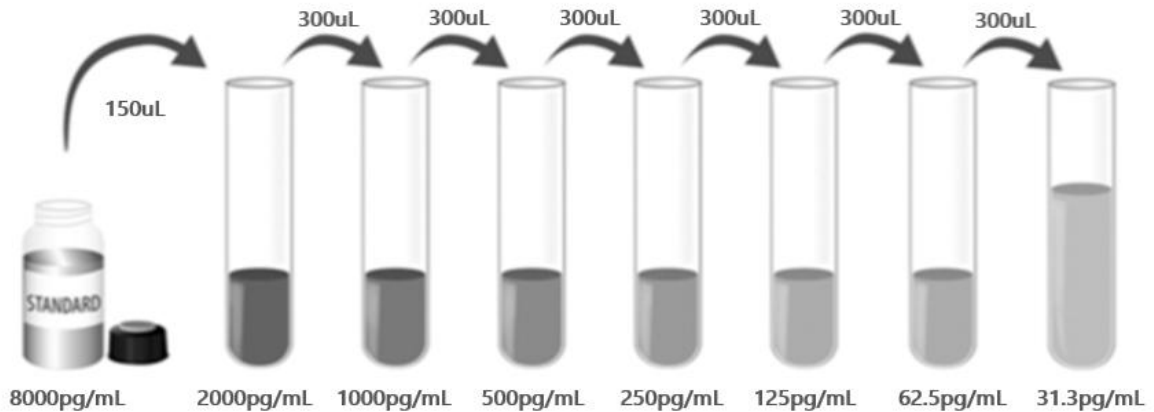
**显色剂:** 按试验所需用量 (100 μL/孔) 将显色液A和显色液B等体积混合, 避光保存, 现用现配, 须在15分钟内使用。

**CCL4/MIP-1β 标准品:** 冻干标准品的重溶体积请参考瓶身标签, 用试剂稀释液 (1×) (用于细胞上清样本) 或标准品稀释液 (1×) (用于血清样本) 重溶冻干标准品。得到浓度为8000 pg/mL标准品母液。轻轻震荡至少15分钟, 使其充分溶解。

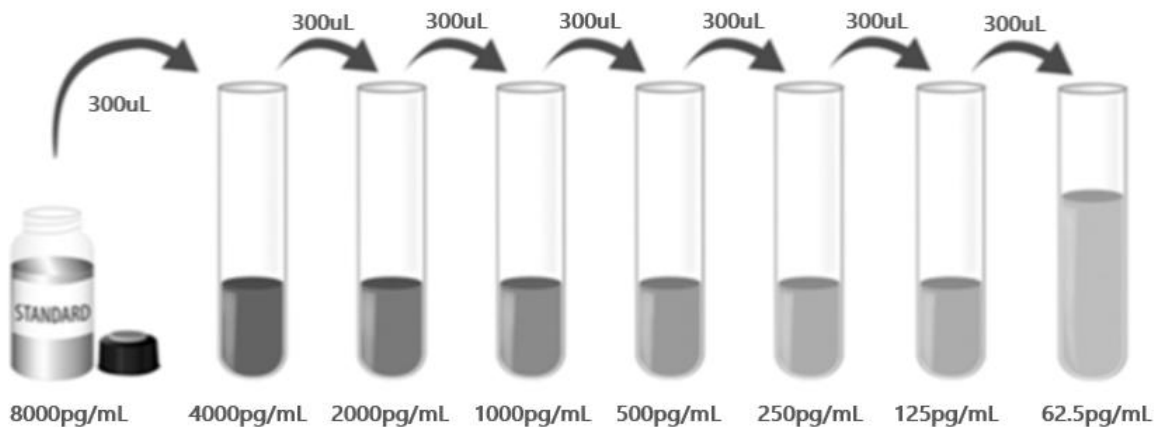
\*如有疑问, 请咨询我们的技术支持。

**细胞上清样本 -** 向 2000 pg/mL稀释管中加入450 μL试剂稀释液 (1×) (用于细胞上清样本), 其余各稀释管中加入300 μL试剂稀释液 (1×)。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。2000 pg/mL管作标准曲线最高点，试剂稀释液（1×）可用作标准品零点（0 pg/mL）。



血清样本 - 各稀释管中加入300  $\mu$ L 标准品稀释液（1×）（用于血清样本）。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。4000 pg/mL管作标准曲线最高点，标准品稀释液（1×）可用作标准品零点（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；
- ◆ 混合后的显色底物在上板前应为无色，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显色底物上板顺序一致；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. 分别将不同浓度标准品，实验样本或者质控品加入相应孔中，每孔100  $\mu\text{L}$ 。用封板膜封住反应孔，**25 $^{\circ}\text{C}$ 恒温箱孵育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；（样本需要稀释，详情参见样本制备部分。）
4. 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  $\mu\text{L}$ ，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；
5. 在每个微孔内加入100  $\mu\text{L}$ 配制好的检测抗体。用封板膜封住反应孔，**25 $^{\circ}\text{C}$ 恒温箱孵育2小时**；
6. 重复第4步洗板操作；
7. 在每个微孔内加入100  $\mu\text{L}$ 稀释好的链霉亲和素- HRP B工作液。用封板膜封住反应孔，**25 $^{\circ}\text{C}$ 恒温箱孵育30分钟，注意避光**；
8. 重复第4步洗板操作；
9. 在每个微孔内加入100  $\mu\text{L}$ 显色剂，**25 $^{\circ}\text{C}$ 恒温箱孵育30分钟，注意避光**；
10. 在每个微孔内加入50  $\mu\text{L}$ 终止液，请轻拍微孔板，使溶液混合均匀。孔内溶液颜色会从蓝色变为黄色；
11. 加入终止液后30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。如果没有使用双波长校正，结果准确度可能会受影响；
12. **计算结果**：将每个标准品和样品的校正吸光度值（ $\text{OD}_{450}-\text{OD}_{540}/\text{OD}_{570}$ ），复孔读数取平均值，然后减去平均零标准品OD值。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。另一种方法是，可以通过绘制标准品浓度做对数与相应OD值对数生成曲线，并通过回归分析确定最佳拟合线。通过样本的OD值，可从标准曲线上得到样本中人CCL4/MIP-1 $\beta$ 的浓度。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

