PRODUCT INFORMATION & MANUAL

Mouse CXCL1/KC Valukine^{TM} ELISA

Catalog Number: VAL619

For the quantitative determination of natural and recombinant
Mouse CXCL1/KC 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 202307.4

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I. BACKGROUND

Mouse CXCL1, also known as KC or N51, was originally identified in fibroblasts as a PDGF-induced immediate early gene that encodes a secretory protein of approximately 8 kDa (1-3). The protein sequence of mouse CXCL1/KC identified it as a member of the alpha (CXC) chemokine family of inflammatory and immunoregulatory cytokines (4). Besides mitogen-stimulated fibroblasts, CXCL1/KC expression can be induced in bacterial or LPS-stimulated peritoneal and lung macrophages, endothelial cells and vascular smooth cells (5). The induction of CXCL1/KC by mitogens has been shown to be inhibited by glucocorticoids (6).

Mouse CXCL1/KC cDNA encodes a 96 amino acid (aa) residue precursor protein from which the amino-terminal 19 aa residues are cleaved to generate the 77 aa residue mature CXCL1/KC (2). The protein sequence of mouse CXCL1/KC shows approximately 63% identity to that of mouse MIP-2, another mouse alpha chemokine. In addition, the protein sequence of CXCL1/KC is approximately 60% identical to the human GROs (2). Like other alpha chemokines, mouse CXCL1/KC is a potent neutrophil attractant and activator. The activities of CXCL1/KC and MIP-2 have been shown to be mediated by the unique mouse IL-8 receptor that shows 71% and 68% aa sequence identity to human IL-8 Rβ and IL-8 Rα, respectively (7, 8). Since an IL-8 homolog has not been identified in mice, it has been suggested that MIP-2 and CXCL1/KC are the functional homologs of IL-8 and may function as the major proinflammatory alpha chemokines in mice. Increased CXCL1/KC expression has been found to be associated with neutrophil influx in various inflammatory conditions (5, 9-11).

II. OVERVIEW

A. PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. An antibody specific for mouse CXCL1/KC has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any mouse CXCL1/KC present is bound by the immobilized antibody. After washing away any unbound substances, a biotinylated detection antibody specific for mouse CXCL1/KC 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, TMB Substrate solution (Chromogenic agent) is added to the wells and color develops in proportion to the amount of CXCL1/KC 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 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 (1×) or Reagent Diluent (1×) 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)	53.0	200.5	716.9	53.5	199.2	715.4
Standard Deviation	1.9	7.3	37.4	1.8	7.0	35.0
CV%	3.5	3.6	5.2	3.4	3.5	4.9

B. RECOVERY

The recovery of mouse CXCL1/KC spiked to different levels throughout the range of the assay in cell culture media was evaluated. The recovery ranged from 93.2-116.1% with an average of 104.3%.

The recovery of mouse CXCL1/KC spiked to different levels throughout the range of the assay in mouse serum was evaluated. The recovery ranged from 92.8-123.0% with an average of 102.4%.

C. SENSITIVITY

The minimum detectable dose (MDD) of mouse CXCL1/KC is typically less than 0.45 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 highly purified *E.coli*-expressed recombinant mouse CXCL1/KC 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 CXCL1/KC and diluted with Reagent Diluent 1× (*for cell culture supernate samples*) or Calibrator Diluent 1× (*for serum samples*) to produce samples with values within the dynamic range of the assay.

Dilution	Average % of Expected	Range (%)
1:2	104.2	97.3-116.9
1:4	105.5	93.8-120.4
1:8	108.3	90.7-124.9
1:16	105.1	86.4-122.0

F. SAMPLE VALUES

Serum - Four mouse serum samples were evaluated for the presence of mouse CXCL1/KC in this assay. All samples measured ranged from 184 to 334 pg/mL with an average of 240.3 pg/mL.

G. SPECIFICITY

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

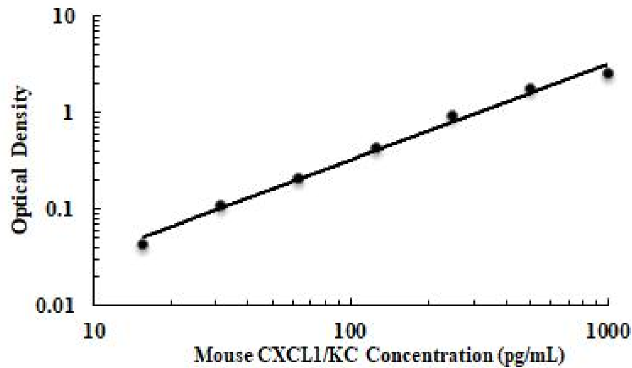
Recombinant mouse	Recombinant human
MIP-1α	GROα
MIP-1β	GROβ
MIP-2	GROγ

A sample containing 3125 pg/mL of recombinant rat CINC-1 reads as 1075 pg/mL (34% cross reactivity).

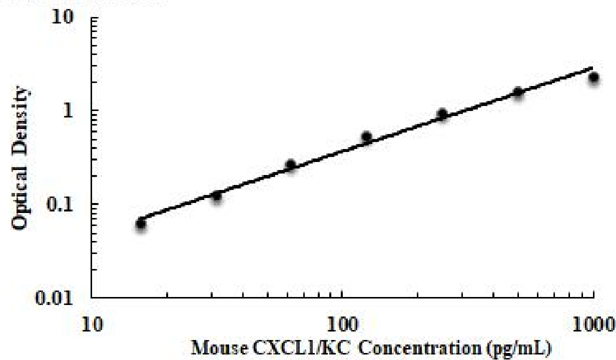
IV. EXPERIMENT

EXAMPLE STANDARD

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



pg/mL	OD	Average	Corrected
0	0.027 0.028	0.028	-
15.6	0.069 0.072	0.071	0.043
31.3	0.134 0.137	0.136	0.108
62.5	0.231 0.242	0.237	0.209
125	0.459 0.464	0.462	0.434
250	0.948 0.963	0.956	0.928
500	1.773 1.813	1.793	1.766
1000	2.531 2.557	2.544	2.517



pg/mL	OD	Average	Corrected
0	0.032 0.033	0.033	-
15.6	0.092 0.096	0.094	0.062
31.3	0.151 0.158	0.155	0.122
62.5	0.301 0.304	0.303	0.270
125	0.560 0.575	0.568	0.535
250	0.967 0.999	0.983	0.951
500	1.619 1.650	1.635	1.602
1000	2.318 2.372	2.345	2.313

V. KIT COMPONENTS AND STORAGE

A. MATERIALS PROVIDED

Parts	Description	Size
Mouse CXCL1/KC Microplate	96 well polystyrene microplate (12 strips of 8 wells) coated with a rat antibody against mouse CXCL1/KC.	1 plate
Mouse CXCL1/KC Standard	Recombinant mouse CXCL1/KC in a buffered protein base; lyophilized. Refer to the vial label for reconstitution volume.	2 vials
Mouse CXCL1/KC Detection Antibody	Biotinylated rat anti-mouse CXCL1/KC antibody, lyophilized. Refer to the vial label for reconstitution volume.	1 vial
Calibrator Diluent (1×)	Concentrated buffered diluent used to dilute standard and samples.	2 vials
Streptavidin-HRP B (40×)	40× Streptavidin conjugated to horseradish peroxidase.	1 vial
Reagent Diluent Concentrate (10×)	A 10× concentrated buffered protein base used to dilute standards, samples, Detection Antibody and HRP.	1 vial
Wash Buffer Concentrate (25×)	A 25× concentrated solution of buffered surfactant with preservatives.	1 vial
TMB Substrate	TMB ELISA Substrate Solution.	1 vial
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="9">Opened/
Reconstituted Reagents</td><td>Streptavidin-HRP B</td><td rowspan="4">May be stored for up to 1 month at 2-8 °C.*</td></tr>
<tr><td>Diluted Wash Solution</td></tr>
<tr><td>TMB Substrate</td></tr>
<tr><td>Stop Solution</td></tr>
<tr><td>Standard</td><td>Prepare fresh for each assay.</td></tr>
<tr><td>Detection Antibody</td><td>Aliquot and store for up to 1 month at -20 °C in a manual defrost freezer. *</td></tr>
<tr><td>Reagent Diluent Concentrate (10×)</td><td>May be stored for up to 1 month at 2-8 °C.*
Use and discard diluted Reagent Diluent (1×). Prepare fresh for each assay.</td></tr>
<tr><td>Calibrator Diluent (1×)</td><td>May be stored for up to 1 month at 2-8 °C.*</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.

D. 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 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 ≤ -20°C. Avoid repeated freeze-thaw cycles. Samples may require dilution with **Reagent 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 x 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** (1×).

B. SAMPLE PREPARATION

Serum samples require a 2-fold dilution prior to the assay. A suggested 2-fold dilution is 100 μL of sample + 100 μL of Calibrator Diluent (1×).

C. REAGENT PREPARATION

Note: *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×).

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

Detection Antibody (1×) - Centrifuge briefly before opening. Reconstitution volume refer to vial label to prepare Detection Antibody (100×). Allow the Detection Antibody to sit for a minimum of 15 minutes with gentle agitation prior to making dilutions. Aliquot and store if needed. Dilute to Detection Antibody (1×) with Reagent Diluent (1×). Prepare at least 15 minutes prior to use.

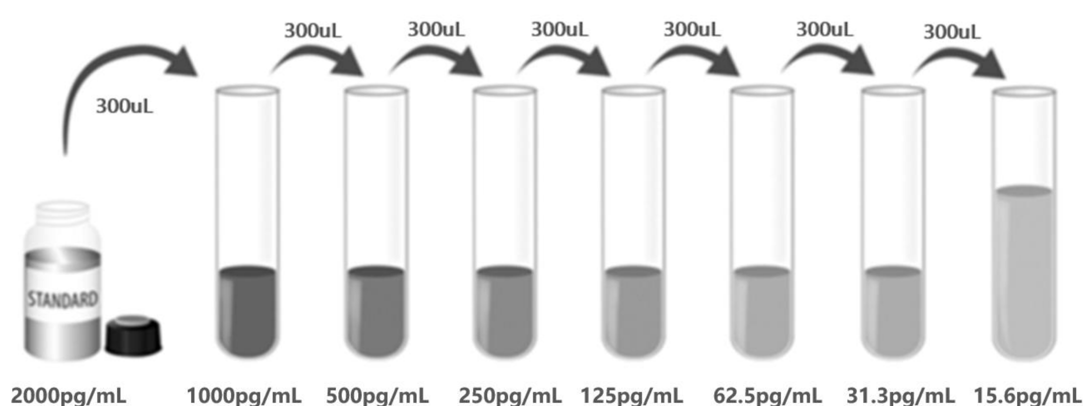
Streptavidin-HRP B (1×) - Dilute to the working concentration specified on the vial label using Reagent Diluent (1×).

Mouse CXCL1/KC Standard - Refer to the vial label for the reconstitution volume*. 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.

Pipette 300 μL of Reagent Diluent 1× (*for cell culture supernate samples*) or Calibrator Diluent 1× (*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 1000 pg/mL standard serves as the high standard. The Reagent Diluent 1× (*for cell culture supernate samples*) or Calibrator Diluent 1× (*for serum 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

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 μL of standard, prepared sample or control 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. (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 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.
5. Add 100 μL of the Detection Antibody (1×) diluted in Reagent Diluent, to each well. Cover with a new adhesive strip and incubate 2 hours at room temperature.
6. Repeat the aspiration/wash as in step 4.
7. Add 100 μL of the working dilution of Streptavidin-HRP B to each well. Cover the plate and incubate for 20 minutes at room temperature. **Avoid placing the plate in direct light**.
8. Repeat the aspiration/wash as in step 4.
9. Add 100 μL of TMB Substrate to each well. Incubate for 20 minutes at room temperature. **Avoid placing the plate in direct light**.
10. Add 50 μL of Stop Solution to each well. Gently tap the plate to ensure thorough mixing.
11. 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.

12. 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 CXCL1/KC 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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7. Lee, J. et al. (1995) J. Immunol. 155:2158.
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9. Tani, M, et al. (1996) J. Clin. Invest. 98:529.
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11. Farone, A. et al. (1995) Am. J. Respir. Cell Mol. Biol. 12:345.

PLATE LAYOUT

Use this plate layout to record standards and samples assayed.

产品信息及操作手册

小鼠 CXCL1/KC Valukine™ ELISA 试剂盒

目录号：VAL619

适用于定量检测天然和重组小鼠 CXCL1/KC 的浓度

科研专用，不可用于临床诊断

Bio-Techne China Co. Ltd

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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 202307.4

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I. 背景

小鼠CXCL1，也被称为KC或N51，最初在成纤维细胞中被鉴定为PDGF诱导的即时早期基因，编码约8 kDa的分泌蛋白(1-3)。小鼠CXCL1/KC蛋白序列被鉴定为炎症和免疫调节细胞因子α(CXC)趋化因子家族的成员(4)。除了有丝分裂原刺激的成纤维细胞外，CXCL1/KC在细菌或LPS刺激的腹腔和肺巨噬细胞、内皮细胞和血管平滑肌细胞中也能诱导表达(5)。糖皮质激素可以抑制有丝分裂原诱导CXCL1/KC (6)。

小鼠CXCL1/KC cDNA编码一个96个氨基酸(aa)残基的前体蛋白，氨基酸末端19个aa残基被切割生成77个aa残基的成熟的CXCL1/KC(2)。小鼠CXCL1/KC的蛋白序列与另一个小鼠α趋化因子MIP-2的同源性约为63%。此外，CXCL1/KC的蛋白序列与人类的GROs大约有60%的同源性(2)。与其他趋化因子一样，小鼠CXCL1/KC是一种强效中性粒细胞引诱剂和激活剂。CXCL1/KC和MIP-2的活性被证明是由唯一的小鼠IL-8受体介导，该受体与人IL-8 Rβ和IL-8 Rα的aa序列同源性分别为71%和68%(7, 8)。由于在小鼠体内尚未发现IL-8同系物，提示MIP-2和CXCL1/KC是IL-8的功能同源物，可能是小鼠主要的促炎性α-趋化因子。研究发现CXCL1/KC表达的增加与各种炎症条件下中性粒细胞内流有关(5, 9 -11)。

II. 概述

A. 检测原理

本实验采用双抗体夹心ELISA法。抗小鼠CXCL1/KC抗体包被于微孔板上，样品和标准品中的小鼠CXCL1/KC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小鼠CXCL1/KC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。洗涤未结合试剂后，加入TMB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，应将样本用试剂稀释液（1×）或标准品稀释液（1×）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，在不同板中分别检测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)	53.0	200.5	716.9	53.5	199.2	715.4
标准差	1.9	7.3	37.4	1.8	7.0	35.0
CV%	3.5	3.6	5.2	3.4	3.5	4.9

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小鼠CXCL1/KC，测定其回收率。回收率范围在93.2-116.1%，平均回收率在104.3%。

在小鼠血清样本中掺入检测范围内不同水平的小鼠CXCL1/KC，测定其回收率。回收率范围在92.8-123.0%，平均回收率在102.4%。

C. 灵敏度

小鼠CXCL1/KC的最低可测剂量（MDD）一般小于0.45 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小鼠CXCL1/KC蛋白所校正。

E. 线性

不同的样本中含有或掺入高浓度的小鼠CXCL1/KC，然后用试剂稀释液（1×）（*用于细胞上清样本*）或标准品稀释液（1×）（*用于小鼠血清样本*）将样本稀释到检测范围内，测定其线性。

稀释倍数	平均值/期待值 (%)	范围 (%)
1:2	104.2	97.3-116.9
1:4	105.5	93.8-120.4
1:8	108.3	90.7-124.9
1:16	105.1	86.4-122.0

F. 样本预值

血清样本 - 使用本试剂盒检测了4份小鼠血清样本中CXCL1/KC的水平。4份样本的检测值范围为184 - 334 pg/mL，平均值为240.3 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。

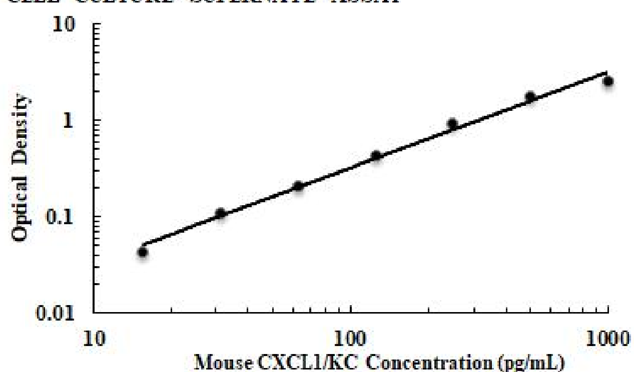
Recombinant mouse	Recombinant human
MIP-1α	GROα
MIP-1β	GROβ
MIP-2	GROγ

大鼠CINC-1重组蛋白浓度为3125 pg/mL时检测值为1075 pg/mL（34%交叉反应）。

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。



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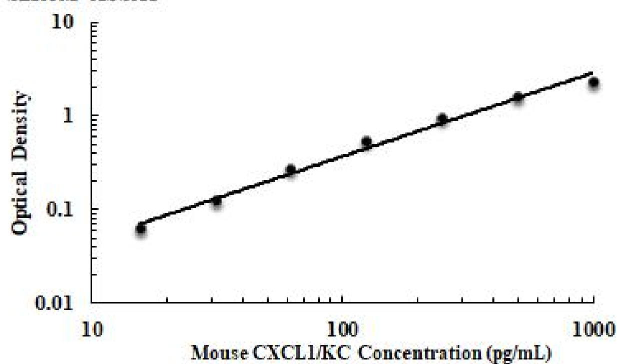
31.3	0.134 0.137	0.136	0.108
62.5	0.231 0.242	0.237	0.209
125	0.459 0.464	0.462	0.434
250	0.948 0.963	0.956	0.928
500	1.773 1.813	1.793	1.766
1000	2.531 2.557	2.544	2.517



pg/mL	OD	Average	Corrected
0	0.032 0.033	0.033	-
15.6	0.092 0.096	0.094	0.062
31.3	0.151 0.158	0.155	0.122
62.5	0.301 0.304	0.303	0.270
125	0.560 0.575	0.568	0.535
250	0.967 0.999	0.983	0.951
500	1.619 1.650	1.635	1.602
1000	2.318 2.372	2.345	2.313

V. 试剂盒组成及储存

A. 试剂盒组成

组成	描述	规格
Mouse CXCL1/KC Microplate	包被大鼠抗小鼠 CXCL1/KC 抗体的 96 孔聚苯乙烯板，8 孔×12 条	1 块板
Mouse CXCL1/KC Standard	标准品（冻干粉），参考瓶身标签进行重溶	2 瓶
Mouse CXCL1/KC Detection antibody	生物素化的 CXCL1/KC 检测抗体，冻干粉，参考瓶身标签进行重溶	1 瓶
Calibrator Diluent (1×)	标准品稀释液（1×）用于稀释标准品和样本	2 瓶
Streptavidin-HRP B (40×)	40×浓缩的链霉亲和素标记的 HRP	1 瓶
Reagent Diluent Concentrate (10×)	浓缩的试剂稀释液（10×）用于标准品、样本、检测抗体和 HRP	1 瓶
Wash Buffer Concentrate (25×)	浓缩洗涤缓冲液（25×）	1 瓶
TMB Substrate	TMB ELISA 底物溶液	1 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℃储存；请在试剂盒有效期内使用</td></tr>
<tr><td rowspan="9">已打开，稀释或重溶的试剂</td><td>链霉亲和素-HRP B</td><td rowspan="4">2-8℃储存，最多 30 天*</td></tr>
<tr><td>洗涤缓冲液（1×）</td></tr>
<tr><td>TMB 底物溶液</td></tr>
<tr><td>终止液</td></tr>
<tr><td>标准品</td><td>使用时新鲜配制*</td></tr>
<tr><td>检测抗体</td><td>分装，-20℃储存，最多 30 天*</td></tr>
<tr><td>试剂稀释液（10×）</td><td>2-8℃储存，最多 30 天*
请每次使用新鲜配制的 1×试剂稀释液，多余的丢弃</td></tr>
<tr><td>标准品稀释液（1×）</td><td>2-8℃储存，最多 30 天*</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℃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

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。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 × 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

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：100μL血清样本+100μL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（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×）。

试剂稀释液（1×）：使用蒸馏水或去离子水稀释配制成试剂稀释液（1×）。

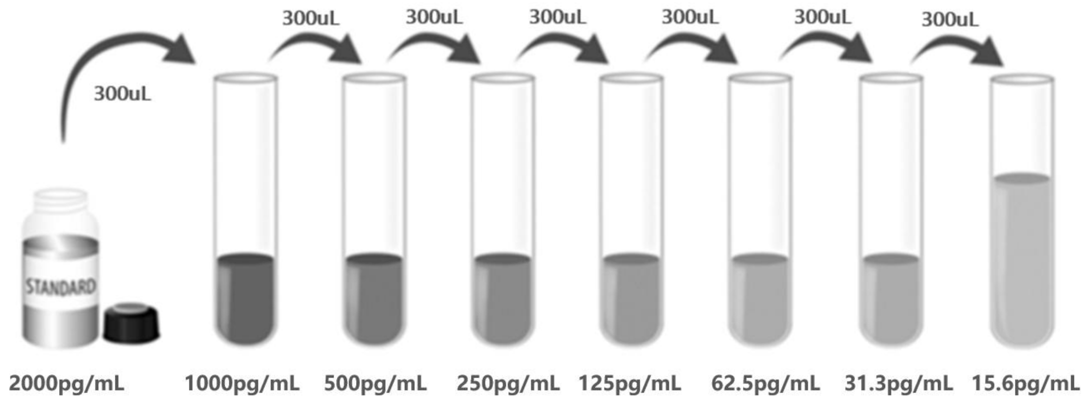
检测抗体（1×）：开盖前请瞬时离心。参考检测抗体瓶标签重溶冻干粉，制备检测抗体（100×）。轻轻震摇至少15分钟，其充分溶解。如有需要分装保存。用试剂稀释液（1×）稀释至检测抗体（1×），至少在使用前15分钟准备。

链霉亲和素- HRP B（1×）：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×）。

小鼠CXCL1/KC标准品：冻干标准品的重溶体积请参考瓶身标签，得到浓度为2000 pg/mL标准品母液。轻轻震摇至少15分钟，使其充分溶解。

*如有疑问，请咨询我们的技术支持。

向各稀释管中加入300 μL试剂稀释液（1×）（*用于细胞上清样本*）或标准品稀释液（1×）（*用于小鼠血清样本*）。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。1000 pg/mL管作标准曲线最高点，试剂稀释液（1×）（*用于细胞上清样本*）或标准品稀释液（1×）（*用于小鼠血清样本*）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；
- TMB底物溶液在上板前应为无色，请避光保存；加入微孔板后，TMB底物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 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；（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 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；
5. 在每个微孔内加入100 μL配制好的检测抗体（1×）。用封板膜封住反应孔，室温孵育2小时；
6. 重复第4步洗板操作；
7. 在每个微孔内加入100 μL稀释好的链霉亲和素- HRP B工作液。用封板膜封住反应孔，室温孵育20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 μL TMB底物溶液，室温孵育20分钟，**注意避光；**
10. 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；
11. 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；
12. **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。数据可以通过绘制小鼠CXCL1/KC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