



## **PRODUCT INFORMATION & MANUAL**

**Mouse GM-CSF Valukine™ ELISA**

**VAL625**

For the quantitative determination of natural and recombinant  
Mouse GM-CSF 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

Version202304.1

## TABLE OF CONTENTS

I. BACKGROUND .....	2
II. OVERVIEW .....	3
III. ADVANTAGES .....	4
IV. EXPERIMENT .....	7
V. KIT COMPONENTS AND STORAGE .....	8
VI. PREPARATION .....	11
VII. ASSAY PROCEDURE .....	13
VIII. REFERENCES .....	15

## I. BACKGROUND

Granulocyte-Macrophage Colony Stimulating Factor (GM-CSF), also known as CSF-2, is a pleiotropic 30 kDa member of the Common beta Chain ( $\beta$ c) cytokine family that also includes IL-3 and IL-5. GM-CSF adopts an  $\alpha$ -helical configuration with two intrachain disulfide bonds. It is secreted by a wide variety of activated immune, mesenchymal, and epithelial cell types and circulates as a variably glycosylated monomer (1, 2). It is upregulated in multiple cell types during inflammation including encephalitogenic T cells (3-5), allergen exposed lung endothelial cells (6), and IgE activated mast cells (7). Mature mouse GM-CSF shares 54% and 69% amino acid sequence identity with human and rat GM-CSF, respectively (8). Rat GM-CSF is known to be active on mouse cells, while mouse GM-CSF has limited activity on rat cells (9).

The high-affinity receptor for GM-CSF is composed of a 50 kDa ligand binding alpha subunit (GM-CSF  $R\alpha$ ) (10) and a 120 kDa signal transducing Common beta Chain ( $\beta$ c) (11, 12). The stoichiometry for the functional GM-CSF receptor is reported to be a 2:2:2 complex of GM-CSF, GM-CSF  $R\alpha$ , and  $\beta$ c (13, 14). Notably, the  $\beta$ c subunit is shared by the receptor complexes for IL-3 and IL-5, and IL-5 may signal through GM-CSF  $R\alpha$  and  $\beta$ c (14). GM-CSF may additionally utilize Syndecan-2 as a co-receptor (15).

A number of functions have been attributed to GM-CSF. It induces monocyte, neutrophil, and eosinophil production from CD34+ stem cell precursors (16, 17). It can act in concert with IL-4 or Flt-3 Ligand to induce the development and maintenance of myeloid and dermal dendritic cells (17-21). It also acts as a neutrophil and dendritic cell chemoattractant (6, 22). GM-CSF promotes Th1 and Th17 cell mediated autoimmune inflammation as well as the inflammatory activation of dendritic cells, microglia, alveolar macrophages, and eosinophils (3-5, 23-27). In addition, it cooperates with G-CSF in promoting tumor cell proliferation and invasion (28).

## II. OVERVIEW

### A. PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. An antibody specific for mouse GM-CSF has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any mouse GM-CSF present is bound by the immobilized antibody. After washing away any unbound substances, a biotinylated detection antibody specific for mouse GM-CSF 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 GM-CSF 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×) 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)	4.8	19.0	72.8	4.8	19.2	73.5
Standard Deviation	0.2	0.4	1.9	0.2	0.4	2.3
CV%	3.4	1.9	2.7	3.4	2.1	3.1

#### B. RECOVERY

The recovery of mouse GM-CSF spiked to different levels throughout the range of the assay in cell culture media was evaluated. The recovery ranged from 83.7 to 93.5% with an average of 90.3%.

The recovery of mouse GM-CSF spiked to different levels throughout the range of the assay in mouse serum was evaluated. The recovery ranged from 83.2 to 88.4% with an average of 86.1%.

#### C. SENSITIVITY

The minimum detectable dose (MDD) of mouse GM-CSF is typically less than 0.26 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 GM-CSF 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 GM-CSF and diluted with Calibrator Diluent (1×) to produce samples with values within the dynamic range of the assay.

Dilution	Average % of Expected	Range (%)
1:2	100.6	98.0-103.7
1:4	102.1	97.5-104.9
1:8	102.1	94.6-106.5
1:16	95.0	91.6-103.4

#### F. SAMPLE VALUES

**Serum** - Four mouse serum samples were evaluated for the presence of mouse GM-CSF in this assay. All samples measured less than the lowest standard, 2.5 pg/mL.

**Cell Culture Supernates** - CTLL-2 ( $2 \times 10^4$  cells/mL) were cultured in RPMI 1640 supplemented with 10% fetal bovine serum, 100 ng/mL MuLL-2, 0.11 g/L sodium pyruvate, 100 U/mL penicillin, and 100 µg/mL streptomycin sulfate. Cells were cultured for 48h. Aliquots of the culture supernates were removed, assayed for mouse GM-CSF, and measured 99.6 pg/mL.

## G. SPECIFICITY

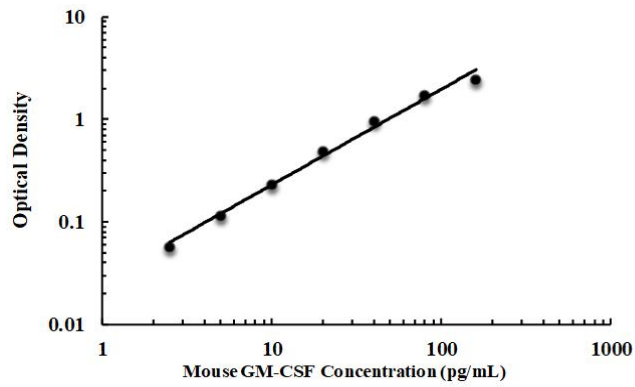
The following factors prepared at 50 ng/mL were assayed and exhibited no cross-reactivity or interference.

<b>Recombinant mouse:</b>	<b>Recombinant human:</b>
G-CSF	G-CSF
IL-3	IL-3
IL-4	IL-4
IL-4 R/Fc Chimera	IL-4R
IL-5	IL-5
M-CSF	M-CSF
<b>Recombinant rat:</b>	GM-CSF
GM-CSF	IL-3 Ra
IL-4	IL-5 Ra
<b>Recombinant porcine:</b>	M-CSF R/Fc Chimera
GM-CSF	
IL-4	

## 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	0.041 0.042	0.042	—
2.5	0.098 0.101	0.099	0.058
5	0.156 0.158	0.157	0.115
10	0.271 0.281	0.276	0.234
20	0.529 0.533	0.531	0.489
40	0.989 1.015	1.002	0.960
80	1.741 1.764	1.752	1.711
160	2.515 2.520	2.517	2.476



## V. KIT COMPONENTS AND STORAGE

### A. MATERIALS PROVIDED

Parts	Description	Size
Mouse GM-CSF Microplate	96 well polystyrene microplate (12 strips of 8 wells) coated with antibody against mouse GM-CSF.	1 plate
Mouse GM-CSF Standard	Recombinant mouse GM-CSF in a buffered protein base; lyophilized. Refer to the vial label for reconstitution volume.	2 vials
Mouse GM-CSF Detection Antibody	Biotinylated mouse GM-CSF antibody, lyophilized. Refer to the vial label for reconstitution volume.	1 vial
Calibrator Diluent Concentrate (10×)	A 10× concentrated buffered protein base used to dilute standard and samples.	1 vial
Detection Antibody Diluent Concentrate (10×)	A 10× concentrated buffered protein base used to dilute detection antibody.	1 vial
Reagent Diluent Concentrate (10×)	A 10× concentrated buffered protein base used to dilute HRP.	1 vial
Streptavidin-HRP A (200×)	200× Streptavidin conjugated to horseradish peroxidase.	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

Unopened Kit	Store at 2-8 °C. Do not use past kit expiration date.	
Opened/ Reconstituted Reagents	Streptavidin-HRP A	May be stored for up to 1 month at 2-8 °C.*
	Diluted Wash Solution	
	TMB Substrate	
	Stop Solution	
	Standard	Prepare fresh for each assay. Standards may be stored for up to 1 month at -20°C *
	Detection Antibody	Aliquot and store for up to 1 month at -20 °C in a manual defrost freezer. *
	Calibrator Diluent Concentrate (10×)	May be stored for up to 1 month at 2-8 °C.* Use and discard diluted Calibrator Diluent (1×). Prepare fresh for each assay.
	Detection Antibody Diluent Concentrate (10×)	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.
	Reagent Diluent Concentrate (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.
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.

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

Mouse 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 (1 $\times$ )** - 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 (1 $\times$ ).

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

**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 $\times$ ).

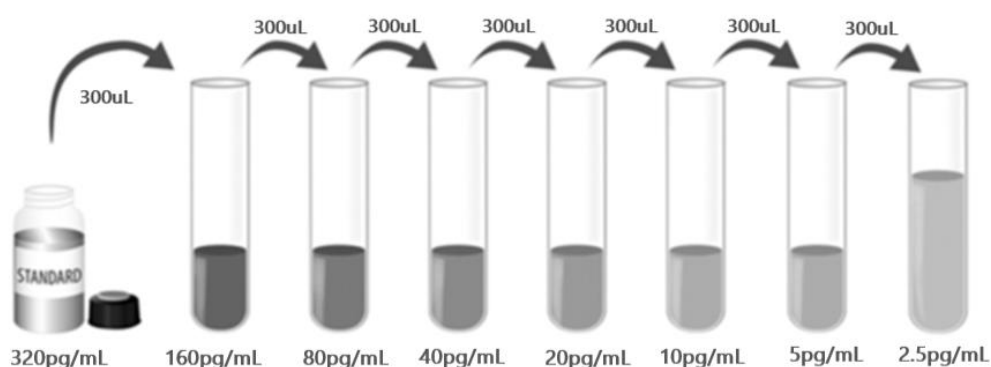
**Detection Antibody (1 $\times$ )** - **Centrifuge briefly before opening. Reconstitution volume refer to vial label to prepare Detection Antibody (100 $\times$ ).** Allow the Detection Antibody to sit for a minimum of 15 minutes with gentle agitation prior to making dilutions. Aliquot and store if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

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

**Mouse GM-CSF Standard - Centrifuge briefly before opening. Refer to the vial label for the reconstitution volume\***. This reconstitution produces a stock solution of 320 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  $\mu$ L of the Calibrator Diluent (1 $\times$ ) into each tube.** Use the stock solution to produce a dilution series (below). Mix each tube thoroughly before the next transfer. The 160 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.
- 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  $\mu$ L of standard or 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. (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 (1 $\times$ ) diluted in Detection Antibody Diluent (1 $\times$ ), to each well. Cover with a new adhesive strip and **incubate for 2 hours at room temperature.**
6. Repeat the aspiration/wash as in step 4.
7. Add 100  $\mu$ L of the working dilution of Streptavidin-HRP A to each well. Cover the plate and **incubate for 30 minutes at room temperature. Avoid placing the plate in direct light.**
8. Repeat the aspiration/wash as in step 4.
9. Add 100  $\mu$ L of TMB Substrate to each well. **Incubate for 20 minutes at room temperature. 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 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, 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 GM-CSF 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.

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## PLATE LAYOUT

Use this plate layout to record standards and samples assayed.

<b>1</b>																	
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	<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

小鼠 GM-CSF Valukine™ ELISA 试剂盒

目录号: **VAL625**

适用于定量检测天然和重组小鼠 GM-CSF 的浓度

科研专用, 不可用于临床诊断

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## 目录

I. 背景 .....	19
II. 概述 .....	20
III. 优势 .....	21
IV. 实验 .....	24
V. 试剂盒组成及储存 .....	25
VI. 实验前准备 .....	27
VII. 操作步骤 .....	29
VIII. 参考文献 .....	30

## I. 背景

粒细胞-巨噬细胞集落刺激因子(GM-CSF), 又称CSF-2, 是常见 $\beta$ 链( $\beta$ c)细胞因子家族的一种30 kDa的多营养成分, 该家族还包括IL-3和IL-5。GM-CSF采用 $\alpha$ -螺旋构型, 具有两个链内二硫键。它是通过多种活化的免疫细胞、间充质细胞和上皮细胞等分泌的, 并以不同糖基化单体的形式循环(1,2)。在炎症期间, GM-CSF在多种细胞类型中上调, 包括致脑炎T细胞(3-5)、过敏原暴露的肺内皮细胞(6)和IgE活化的肥大细胞(7)。成熟小鼠GM-CSF与人和大鼠GM-CSF的氨基酸序列分别有54%和69%的同源性(8)。大鼠GM-CSF对小鼠细胞具有活性, 然而小鼠GM-CSF在大鼠细胞内的活性是有限的(9)。

GM-CSF的高亲和力受体由50 kDa配体结合 $\alpha$ 亚基(GM-CSF R $\alpha$ )(10)和120 kDa信号转导共同 $\beta$ 链( $\beta$ c)组成(11,12)。据报道, 功能性GM-CSF受体的化学计量是由GM-CSF, GM-CSF R $\alpha$ 和GM-CSF  $\beta$ c组成的2:2:2复合物(13,14)。值得注意的是,  $\beta$ c亚基是由IL-3和IL-5的受体复合物共享的, IL-5可能通过GM-CSF R $\alpha$ 和 $\beta$ c传递信号(14)。GM-CSF还可以利用Syndecan-2作为辅助受体(15)。

GM-CSF具有多种功能。它诱导CD34+干细胞前体产生单核细胞、中性粒细胞和嗜酸性粒细胞(16,17)。它还可以与IL-4或Flt-3配体协同作用, 诱导髓细胞和真皮树突状细胞的发育和维持(17-21)。它还充当中性粒细胞和树突状细胞的化学趋化剂(6,22)。GM-CSF促进Th1和Th17细胞介导的自身免疫炎症, 以及树突状细胞、小胶质细胞、肺泡巨噬细胞和嗜酸性粒细胞的炎症激活(3-5,23-27)。此外, 它还协同G-CSF促进肿瘤细胞增殖和侵袭(28)。

## II. 概述

### A. 检测原理

本实验采用双抗体夹心ELISA法。抗小鼠GM-CSF抗体包被于微孔板上，样本和标准品中的小鼠GM-CSF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GM-CSF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)	4.8	19.0	72.8	4.8	19.2	73.5
标准差	0.2	0.4	1.9	0.2	0.4	2.3
CV%	3.4	1.9	2.7	3.4	2.1	3.1

#### 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GM-CSF，测定其回收率。回收率范围在83.7-93.5%，平均回收率在90.3%。

在小鼠血清样本中掺入检测范围内不同水平的小鼠GM-CSF，测定其回收率。回收率范围在83.2-88.4%，平均回收率在86.1%。

#### C. 灵敏度

小鼠GM-CSF的最低可测剂量（MDD）一般小于0.26 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GM-CSF 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GM-CSF，然后用标准品稀释液（1×）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	100.6	98.0-103.7
1:4	102.1	97.5-104.9
1:8	102.1	94.6-106.5
1:16	95.0	91.6-103.4

## 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GM-CSF的水平。4份样本的检测值均低于最低标准品，2.5 pg/mL。

**细胞上清样本** - CTLL-2（ $2 \times 10^4$  cells/mL）培养于含有100ng/ml MuIL-2和0.11g/L丙酮酸钠和10%胎牛血清的RPMI-1640培养基中，加100 U/mL青霉素和100  $\mu$ g/mL硫酸链霉素。培养48h后，得到的细胞培养上清，测定小鼠GM-CSF的结果为99.6 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。

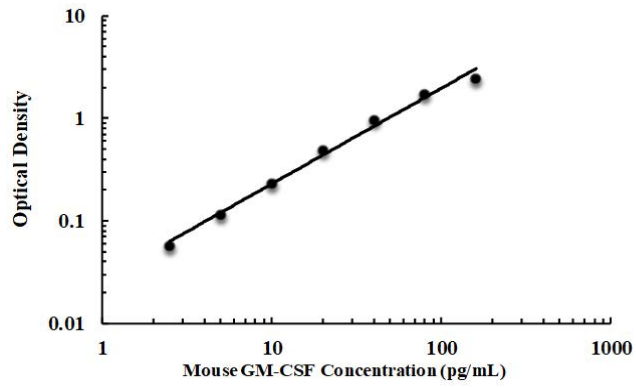
<b>Recombinant mouse:</b>	<b>Recombinant human:</b>
G-CSF	G-CSF
IL-3	IL-3
IL-4	IL-4
IL-4 R/Fc Chimera	IL-4R
IL-5	IL-5
M-CSF	M-CSF
<b>Recombinant rat:</b>	GM-CSF
GM-CSF	IL-3 Ra
IL-4	IL-5 Ra
<b>Recombinant porcine:</b>	M-CSF R/Fc Chimera
GM-CSF	
IL-4	



## 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	0.041 0.042	0.042	—
2.5	0.098 0.101	0.099	0.058
5	0.156 0.158	0.157	0.115
10	0.271 0.281	0.276	0.234
20	0.529 0.533	0.531	0.489
40	0.989 1.015	1.002	0.960
80	1.741 1.764	1.752	1.711
160	2.515 2.520	2.517	2.476

## V. 试剂盒组成及储存

### A. 试剂盒组成

组成	描述	规格
Mouse GM-CSF Microplate	包被抗小鼠 GM-CSF 抗体的 96 孔聚苯乙烯板，8 孔×12 条	1 块板
Mouse GM-CSF Standard	标准品（冻干粉），参考瓶身标签进行重溶	2 瓶
Mouse GM-CSF Detection Antibody	生物素化的小鼠 GM-CSF 检测抗体，冻干粉，参考瓶身标签进行重溶	1 瓶
Calibrator Diluent Concentrate (10×)	浓缩的标准品稀释液（10×）用于稀释标准品和样本	1 瓶
Detection Antibody Diluent Concentrate (10×)	浓缩的检测抗体稀释液（10×）用于稀释检测抗体	1 瓶
Reagent Diluent Concentrate (10×)	浓缩的试剂稀释液（10×）用于稀释 HRP	1 瓶
Streptavidin-HRP A (200×)	200×浓缩的链霉亲和素标记的 HRP	1 瓶
Wash Buffer Concentrate (25×)	浓缩洗涤缓冲液（25×）	1 瓶
TMB Substrate	TMB 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

未开封试剂盒	2-8℃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 A	2-8℃储存，最多 30 天*
	洗涤缓冲液（1×）	
	TMB 底物溶液	
	终止液	
	标准品	使用时新鲜配制* 标准品-20℃储存，最多 30 天*
	检测抗体	分装， -20℃储存，最多 30 天*
	标准品稀释液（10×）	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
	检测抗体稀释液（10×）	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
	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
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

## 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。样本可能需要用标准品稀释液（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标准品稀释液（1×）2倍稀释后进行检测，例如：100 μL样本 +100 μL标准品稀释液（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（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浓缩洗涤液用去离子水或蒸馏水稀释配制成500mL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稀释配制成标准品稀释液（1×）。

**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×）：**使用蒸馏水或去离子水稀释配制成检测抗体稀释液（1×）。

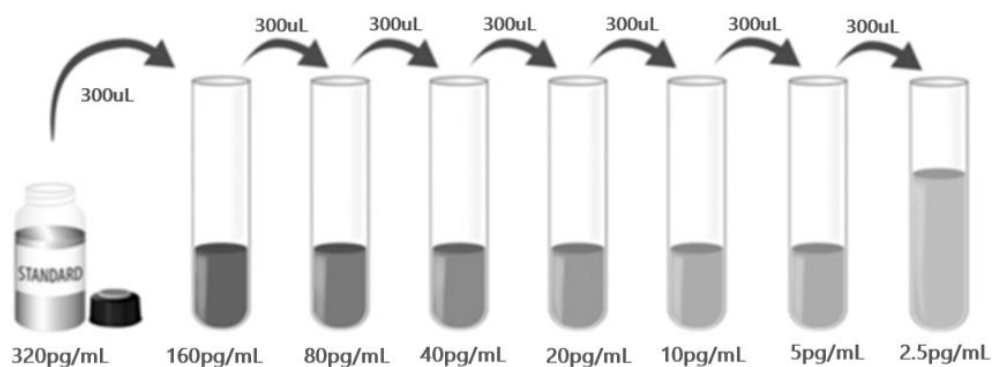
**检测抗体（1×）：**开盖前请瞬时离心。参考检测抗体瓶标签重溶冻干粉，制备检测抗体（100×）。轻轻震荡至少15分钟，其充分溶解。如有需要分装保存。用检测抗体稀释液（1×）稀释至检测抗体（1×），至少在使用前15分钟准备。

**链霉亲和素-HRP A（1×）：**开盖前请瞬时离心。用试剂稀释液（1×）将链霉亲和素-HRP A（200×）稀释至工作浓度链霉亲和素-HRP A（1×）。

**小鼠GM-CSF标准品：**开盖前请瞬时离心。冻干标准品的重溶体积请参考瓶身标签，得到浓度为320 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。

向各稀释管中加入300  $\mu\text{L}$ 标准品稀释液(1 $\times$ )。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。160  $\text{pg/mL}$ 管作标准曲线最高点,标准品稀释液(1 $\times$ )可用作标准品零点(0  $\text{pg/mL}$ )。



#### 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. 分别将标准品或者样本加入相应孔中，每孔100  $\mu\text{L}$ 。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  $\mu\text{L}$ ，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（1 $\times$ ）。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  $\mu\text{L}$ 稀释好的链霉亲和素- HRP A工作液。用封板膜封住反应孔，**室温孵育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  $\mu\text{L}$  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  $\mu\text{L}$ 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（OD450-OD540/OD570），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小鼠GM-CSF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。

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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

