PRODUCT INFORMATION & MANUAL

Mouse IL-4 Valukine™ ELISA

Catalog Number: VAL603

For the quantitative determination of natural and recombinant mouse IL-4 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 202408.5

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I. BACKGROUND

Interleukin-4 (IL-4), also known as B cell-stimulatory factor-1, is an approximately 18-20 kDa monomeric cytokine that displays pleiotropic effects during immune responses (1-4). Mouse IL-4 is synthesized as a 140-amino acid (aa) precursor with a 20 aa signal sequence and a 120 aa mature chain. The cytokine contains three potential sites for N-linked glycosylation, three intrachain disulfide bonds, and adopts a bundled four α-helix structure (5). Analogous to human, mouse IL-4 has a reported alternative splicing short form. Unlike in human, this form is suggested to be very minor in expression and of questionable significance (6). Mature mouse IL-4 shares 43%, and 63% aa sequence identity with human and rat IL-4, respectively. Research has shown that human, mouse, and rat IL-4 are all species-specific in their activities (7-9). IL-4 is expressed by Th2-biased $CD4^+$ T cells, basophils, mast cells, NKT and γδ T cells, and eosinophils (1-2, 10).

IL-4 is a key player in the type 2 immune response as it promotes Th2 differentiation and B cell commitment to the immunoglobulin G1 and immunoglobulin E isotypes (11). IL-4 initiates T helper type 2 (Th2) differentiation by binding to either a type I or type II receptor complex that contains the IL-4 Rα subunit coupled to either the chain or the IL-13Rα1, respectively (12). Phosphorylation of the cytoplasmic C-terminal tails by the Janus Kinase (JAK) family of tyrosine kinases results from the heterodimerization of the IL-4 receptor on the cell surface (12). This then leads to the recruitment and phosphorylation of STAT6 (12). Following the phosphorylation of STAT6, conformational changes take place leading to dimerization, nuclear translocation, DNA binding and transcriptional activation of several target genes including the genes for IL-4, IL-5, and IL-13, and the Th2 specific factors GATA3 and c-Maf (12).

Functionally, IL-4 promotes cell proliferation and survival, immunoglobulin class switch to IgG1 and IgE in mouse B cells, priming and chemotaxis of mast cells, eosinophils, and basophils, an acquisition of the Th2 phenotype by naïve $CD4^+$ cells, and the proliferation and activation of epithelial cells (13-16). IL-4 also is a significant cytokine in tumor immunology (17). Researchers found in early mouse experiments that IL-4 exhibited potent anti-tumor ability. The mice rejected IL-4 producing tumors and developed long-lasting anti-tumor immunity (17). This is perhaps due to IL-4's antiangiogenic effect and/or its ability to activate select $CD8^+$ T cells. Paradoxically, new evidence shows that IL-4 is a tumor-promoting molecule, and thus a cytokine with opposite effects (17). This is possible due to the IL-4 induced upregulation of antiapoptotic molecules in tumor cells, and the downregulation of cytolytic molecules on $CD8^+$ T cells. In addition, tumor produced IL-4 is also suggested to act on local tumor-associated-macrophages (TAMs), inducing the secretion of cathepsins that promote cell migration (18).

II. OVERVIEW

A. PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. An antibody specific for mouse IL-4 has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any mouse IL-4 present is bound by the immobilized antibody. After washing away any unbound substances, an enzyme-linked antibody specific for mouse IL-4 is added to the wells. Following a wash to remove any unbound antibody-enzyme reagent, TMB substrate solution (Chromogenic agent) is added to the wells and color develops in proportion to the amount of mouse IL-4 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)

Two 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	1	2	3
Mean (pg/mL)	13.8	260	15.3	49.5	238
Standard Deviation	1.1	14.8	1.9	5.3	15.8
CV%	8.0	5.7	12.4	10.7	6.7

B. RECOVERY

The recovery of mouse IL-4 spiked to different levels throughout the range of the assay in cell culture media was evaluated. The recovery ranged from 75.0 to 112.0% with an average of 90.0%.

The recovery of mouse IL-4 spiked to different levels throughout the range of the assay in mouse serum was evaluated. The recovery ranged from 71.8 to 84.3% with an average of 80.4%.

C. SENSITIVITY

The minimum detectable dose (MDD) of mouse IL-4 is typically less than 2.3 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 IL-4 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 IL-4 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	102	96 - 105
1:4	103	98 - 108
1:8	109	103 - 116
1:16	102	98 - 105

F. SAMPLE VALUES

Cell Culture Supernates - Two spleen organ tissues from a mouse were homogenized and seeded in 100 mL of RPMI1640 supplemented with 10% fetal bovine serum, 2 mM L-glutamine, 100 U/mL penicillin, 100 µg/mL streptomycin sulfate, and 10 µg/mL Con A for 2 days. The cell culture supernate was assayed for mouse IL-4 and measured 82.8 pg/mL.

EL-4 cells (Mouse thymoma) were seeded at $2x10^5$ cells/mL and cultured for 4 days in 100 mL of DMEM supplemented with 10% horse serum, 10 µg/mL PHA and 10 ng/mL PMA. The cell culture supernate was assayed for mouse IL-4 and measured 3140 pg/mL.

Mouse serum - Four mouse serum samples were evaluated for the presence of IL-4 in this assay. All samples measured below the lowest standard, 7.8 pg/mL.

G. SPECIFICITY

This assay recognizes both natural and recombinant mouse IL-4. The following factors were prepared at 50 ng/mL and assayed for cross-reactivity. Preparations of the following factors at 50 ng/mL in a mid-range recombinant mouse IL-4 control were assayed for interference. No significant cross-reactivity or interference was observed.

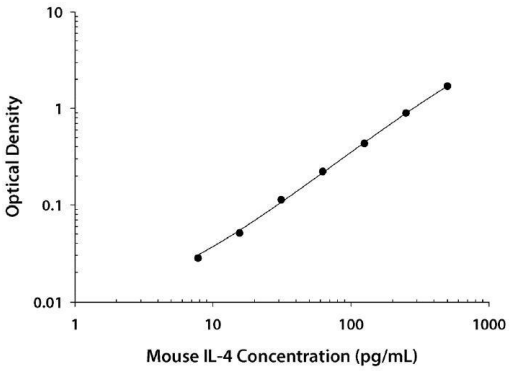
Recombinant mouse	
IL-2	IL-5
IL-3	IL-13
IL-4 I1	

At concentration greater than 10 ng/mL, mouse IL-4 R interferes in the assay.

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	0.070 0.065	0.068	—
7.8	0.094 0.097	0.096	0.028
15.6	0.120 0.118	0.119	0.051
31.3	0.179 0.183	0.181	0.113
62.5	0.293 0.287	0.290	0.222
125	0.508 0.492	0.500	0.432
250	0.960 0.962	0.961	0.893
500	1.775 1.761	1.768	1.700

V. KIT COMPONENTS AND STORAGE

A. MATERIALS PROVIDED

Parts	Description	Size
Mouse IL-4 Microplate	96 well polystyrene microplate (12 strips of 8 wells) coated with an antibody against mouse IL-4	1 plate
Mouse IL-4 Conjugate	Antibody against mouse IL-4 conjugated to horseradish peroxidase	1 vial
Mouse IL-4 Standard	Recombinant mouse IL-4 in a buffered protein base; lyophilized	1 vial
Calibrator Diluent (5×)/RD5P	A 5× concentrated 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	1 vial
Stop Solution	Diluted hydrochloric acid solution	1 vial
Plate Covers	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="6">Opened/ Reconstituted Reagents</td><td>Wash Buffer (1×)</td><td rowspan="4">May be stored for up to 1 month at 2-8°C.*</td></tr>
<tr><td>Stop Solution</td></tr>
<tr><td>Conjugate</td></tr>
<tr><td>TMB Substrate</td></tr>
<tr><td>Standard</td><td>Aliquot and store for up to 1 month at -20°C in a manual defrost freezer.* Avoid repeated freeze-thaw cycles.</td></tr>
<tr><td>Calibrator Diluent (5×)/RD5P</td><td>May be stored for up to 1 month at 2-8 °C.*
Use and discard diluted Calibrator Diluent (1×). Prepare fresh for each assay.</td></tr>
<tr><td>Microplate Wells</td><td>Return unused wells to the oil ouch 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 eye, hand, face, and clothing protection when using this material.

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 Calibrator Diluent (1×).

Serum - Use a serum separator tube (SST) and allow samples to clot for 30 minutes at room temperature before centrifugation for 15 minutes at 1000 × g. Remove serum and assay immediately or aliquot and store samples at ≤ -20°C. Avoid repeated freeze-thaw cycles. Samples may require dilution with Calibrator Diluent (1×).

B. SAMPLE PREPARATION

Mouse serum samples recommend a 5-fold dilution. A suggested 5-fold dilution is 40 µL of sample + 160 µL of Calibrator Diluent (1×). Optimal dilutions should be determined by the end user.

C. REAGENT PREPARATION

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

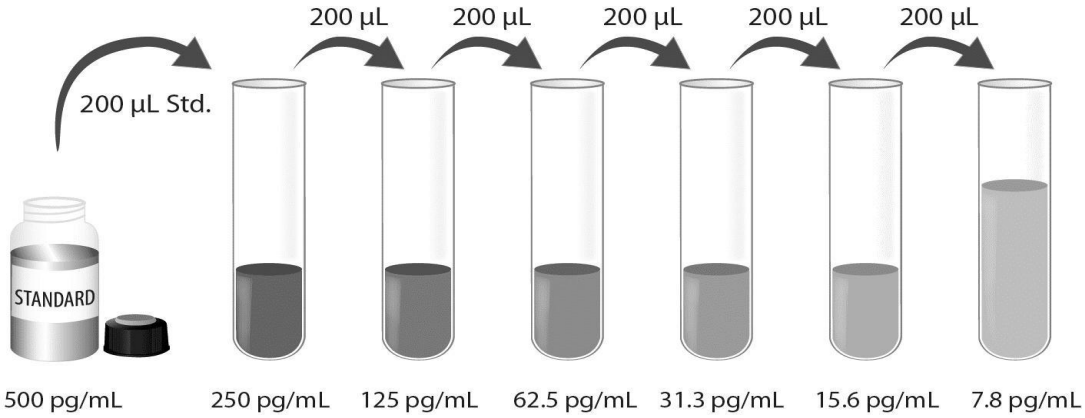
Wash Buffer (1×) - If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. Dilute 20 mL of Wash Buffer Concentrate (25×) into deionized or distilled water to prepare 500 mL of Wash Buffer (1×).

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

Mouse IL-4 Standard - Refer to the vial label for reconstitution volume*. This reconstitution produces a stock solution of 500 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 200 µL of Calibrator Diluent (1×) into each tube. Use the stock solution to produce a dilution series (below). Mix each tube thoroughly before the next transfer. The undiluted standard 500 pg/mL serves as the high standard. The Calibrator Diluent (1×) 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 50 µL of Calibrator Diluent (1×) to each well.
4. Add 50 µL of Standard and prepared sample per well. Cover with the adhesive strip provided. **Incubate for 2 hours at room temperature.** A plate layout is provided for a record of standards and samples assayed. (Samples may require dilution. See Sample Preparation section.)
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 IL-4 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 and sample and subtract the average zero standard optical density. 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 IL-4 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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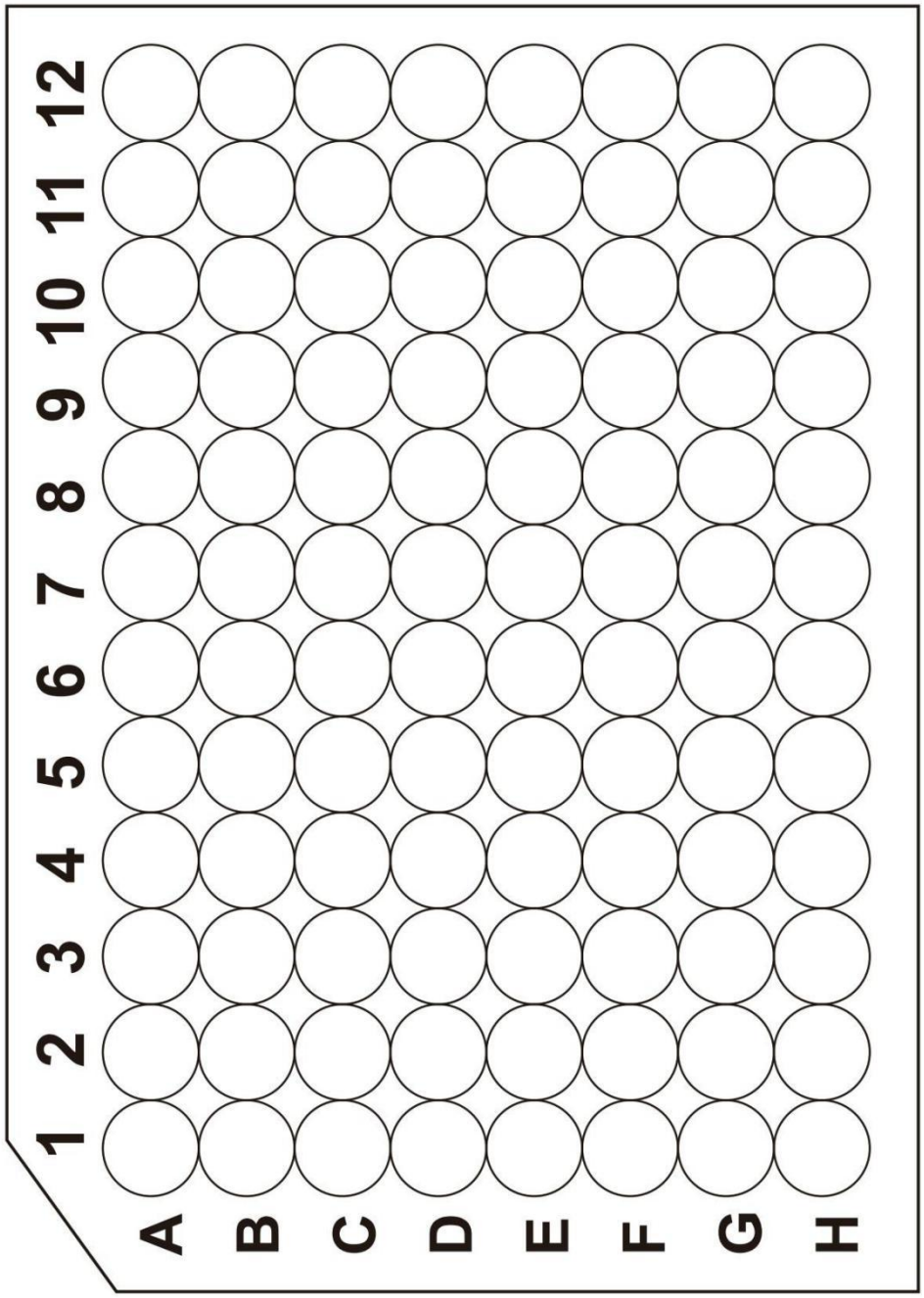
PLATE LAYOUT

Use this plate layout to record standards and samples assayed.



产品信息及操作手册

小鼠 IL-4 Valukine™ ELISA 试剂盒

目录号：VAL603

适用于定量检测天然和重组小鼠 IL-4 的浓度

科研专用，不可用于临床诊断

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版本号 202408.5

目录

I. 背景

白细胞介素4（IL-4），又称为B细胞刺激因子1，是一个约为18-20kDa的单体细胞因子，在免疫反应中呈多向性效应（1-4）。小鼠IL-4的前体有140个氨基酸，包括一个20个氨基酸的信号肽和一个120氨基酸的成熟链。IL-4含有3个N链接糖基化潜在位点，3个链内二硫键，其结构为一个捆绑的四α-螺旋体（5）。与人IL-4相似，小鼠IL-4有一个小的选择性剪切体。与人IL-4不同的是，此剪切体被认为表达很低，意义不大（6）。成熟的小鼠IL-4氨基酸序列与人和大鼠IL-4分别有43%和63%同源性。研究显示，人、小鼠和大鼠IL-4的活性均属于种属特异性（7-9）。IL-4由趋向Th2的$CD4^+$ T细胞、嗜碱性粒细胞、肥大细胞、NK T细胞、γδT细胞和嗜酸性细胞表达（1-2, 10）。

IL-4在2型免疫反应中起关键作用，能促进Th2分化和B细胞分泌免疫球蛋白G1和免疫球蛋白E亚型（11）。IL-4通过结合I型或II型受体复合物引起Th2分化，该复合物含有分别与γ链或IL13Rα1偶联IL-4受体α亚基（12）。通过细胞表面IL-4受体的异二聚体化，激活JAK族蛋白络氨酸激酶，引起其细胞质内C端磷酸化（12）。从而引发STAT6的招募和磷酸化（12）。随着STAT6的磷酸化、构象发生变化，导致STAT6的二聚体化、核转位、与靶基因DNA结合和转录激活，这些靶基因包括IL-4、IL-5、IL-13以及Th2特异性因子GATA3和c-Maf等（12）。

在功能上，IL-4促进小鼠B细胞增殖、生存和免疫球蛋白类转换为IgG1和IgE，促进肥大细胞、嗜酸性粒细胞和嗜碱性粒细胞的启动和趋化，促进幼稚$CD4^+$细胞获得Th2表型，以及促进上皮细胞的增殖和活化（13-16）。在肿瘤免疫学方面，IL-4也是一个重要细胞因子（17）。在早期小鼠实验中，研究人员发现IL-4具有强大的抗肿瘤能力。小鼠排斥表达IL-4的肿瘤，形成长期的抗肿瘤免疫（17）。这可能是由于IL-4具有抗血管生成作用或激活、选择$CD8^+$ T细胞的能力。矛盾的是，新的证据显示IL-4是一个促肿瘤分子，因此IL-4是一个具有反向作用的细胞因子（17）。可能的解释是，IL-4诱导肿瘤细胞中的抵抗凋亡分子上调，和$CD8^+$ T细胞中的溶细胞分子下调。另外，肿瘤产生的IL-4也被认为可作用于局部肿瘤相关巨噬细胞（TAMs），从而诱导促进细胞迁移的组织蛋白酶的分泌（18）。

II. 概述

A. 检测原理

本实验采用双抗体夹心ELISA法。抗小鼠IL-4抗体包被于微孔板上，样品和标准品中的小鼠IL-4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IL-4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）

已知浓度的两个样本，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	1	2	3
平均值 (pg/mL)	13.8	260	15.3	49.5	238
标准差	1.1	14.8	1.9	5.3	15.8
CV%	8.0	5.7	12.4	10.7	6.7

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小鼠IL-4，测定其回收率。回收率范围在75.0-112.0%，平均回收率在90.0%。

在小鼠血清样本中掺入检测范围内不同水平的小鼠IL-4，测定其回收率。回收率范围在71.8-84.3%，平均回收率在80.4%。

C. 灵敏度

小鼠IL-4的最低可测剂量（MDD）一般小于2.3 pg/mL。

最低可测值是根据20个标准曲线零点吸光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R&D Systems®生产的大肠杆菌表达的高纯度重组小鼠IL-4蛋白校正。

E. 线性

不同的样本中含有或掺入高浓度的小鼠IL-4，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	102	96 - 105
1:4	103	98 - 108
1:8	109	103 - 116
1:16	102	98 - 105

F. 样本预值

细胞培养上清液 - 从两个小鼠脾脏组织匀浆，得到的原代细胞培养于100 mL的RPMI1640培养基中，细胞培养基含有10%胎牛血清2 mML-谷氨酰氨，100 U/mL青霉素，100 µg/mL硫酸链霉素，10 µg/mL ConA，培养两天。取细胞培养上清液测定小鼠IL-4含量，结果为82.8 pg/mL。

EL-4细胞（小鼠胸腺瘤）以2×10^5细胞/mL铺板，培养四天。培养基100 mL的DMEM含有10%马血清、10 µg/mL PHA和10 ng/mL PMA。取细胞培养上清液测定小鼠IL-4含量，结果为3140 pg/mL。

小鼠血清样本 - 使用本试剂盒检测了4份小鼠血清样本中IL-4的水平。所有样本的检测值均低于最低标准品，7.8 pg/mL。

G. 特异性

此ELISA法可检测天然及重组小鼠IL-4蛋白。将以下因子用标准品稀释液（1×）配制成50 ng/mL的浓度来检测与小鼠IL-4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IL-4对照品中，来检测对小鼠IL-4的干扰。没有观察到明显的交叉反应或干扰。

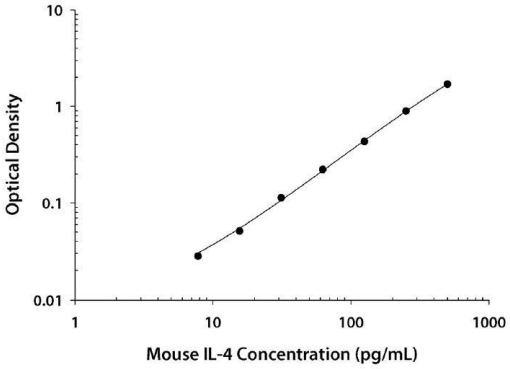
Recombinant mouse	
IL-2	IL-5
IL-3	IL-13
IL-4 I1	

浓度大于10 ng/mL时，小鼠IL-4R会对检测造成影响。

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	0.070 0.065	0.068	—
7.8	0.094 0.097	0.096	0.028
15.6	0.120 0.118	0.119	0.051
31.3	0.179 0.183	0.181	0.113
62.5	0.293 0.287	0.290	0.222
125	0.508 0.492	0.500	0.432
250	0.960 0.962	0.961	0.893
500	1.775 1.761	1.768	1.700

V. 试剂盒组成及储存

A. 试剂盒组成

组成	描述	规格
Mouse IL-4 Microplate	包被抗小鼠 IL-4 抗体的 96 孔聚苯乙烯板，8 孔×12 条	1 块板
Mouse IL-4 Conjugate	酶标检测抗小鼠 IL-4 抗体	1 瓶
Mouse IL-4 Standard	小鼠 IL-4 标准品（冻干），参考瓶身标签进行重溶	1 瓶
Calibrator Diluent (5×)/RD5P	浓缩的标准品稀释液（5×）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	1 瓶
Stop Solution	终止液	1 瓶
Plate Covers	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="6">已打开，稀释或重溶的试剂</td><td>洗涤液（1×）</td><td rowspan="4">2-8℃储存，最多 30 天*</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></tr>
<tr><td>标准品</td><td>分装，-20℃冰箱储存 30 天*；避免反复冻融</td></tr>
<tr><td>稀释液（5×）/RD5P</td><td>2-8℃储存，最多 30 天*
请每次使用新鲜配制的稀释液（1×），多余的丢弃</td></tr>
<tr><td>包被的微孔板条</td><td>将未用的板条放回带有干燥剂的铝箔袋内，密封；2-8℃储存，最多 30 天*</td></tr>
</table>

*必须在试剂盒有效期

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。

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 × 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

小鼠血清样本建议5倍稀释后进行检测，即40 µL血清+160 µL标准品稀释液（1×）。最佳稀释度应由最终用户确定。

C. 检测前准备工作

使用前请将所有试剂放置于室温

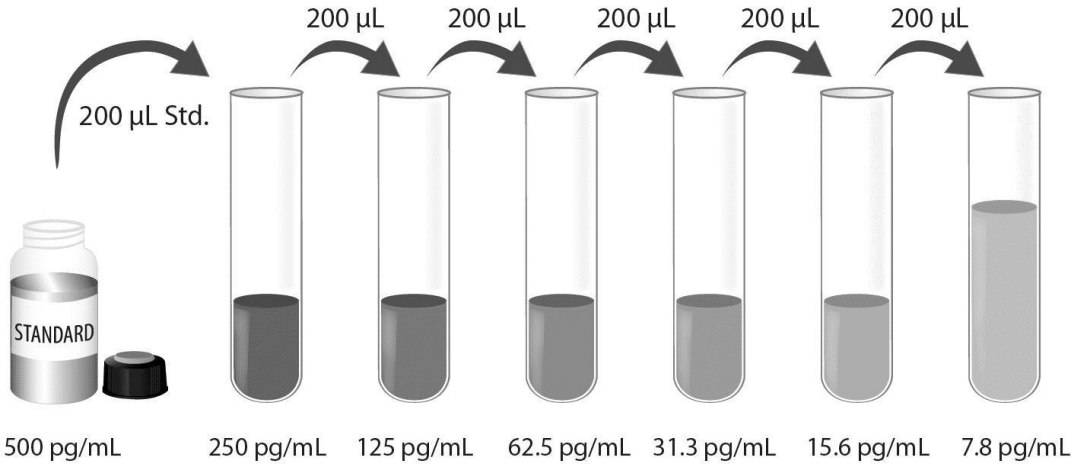
洗涤液（1×）：从冰箱中取出的浓缩洗涤液可能有结晶，属于正常现象；放置室温，轻摇混匀， 待结晶完全溶解后再配制洗涤液。可将20 mL浓缩洗涤液（25×）用蒸馏水或去离子水稀释配制成500 mL工作浓度的洗涤液（1×）。

标准品稀释液（1×）：使用去离子水或蒸馏水稀释配制成标准品稀释液（1×）。

小鼠IL-4标准品：参照标准品瓶身注明的方式重溶冻干标准品。得到浓度为500 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。

每个稀释管中加入200 µL标准品稀释液（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。没有稀释的标准品母液可用作标准曲线最高点（500 pg/mL），标准品稀释液（1×）可用作标准曲线零点（0 pg/mL）。



D. 技术小提示

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

VII.操作步骤

使用前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. 在每个微孔中加入50 µL标准品稀释液（1×）；
4. 分别将不同浓度标准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；（样本需要稀释，详情参见样本制备部分。）
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IL-4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