PRODUCT INFORMATION & MANUAL

Mouse IL-4 Valukine™ ELISA

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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Novus kits are guaranteed for 3 months from date of receipt

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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. A monoclonal 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 polyclonal antibody specific for mouse IL-4 is added to the wells. Following a wash to remove any unbound antibody-enzyme reagent, a substrate solution 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 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 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.

C. TECHNICAL HINTS

- When mixing or reconstituting protein solutions, always avoid foaming.
- To avoid cross-contamination, change pipette tips between additions of each standard level, between sample additions, and between reagent additions. Also, use separate reservoirs for each reagent.
- 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.

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
n	20	20	20	20	20
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 75-112% with an average of 90%.

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 71.8-84.3% 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 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 RPMI 1640 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.

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

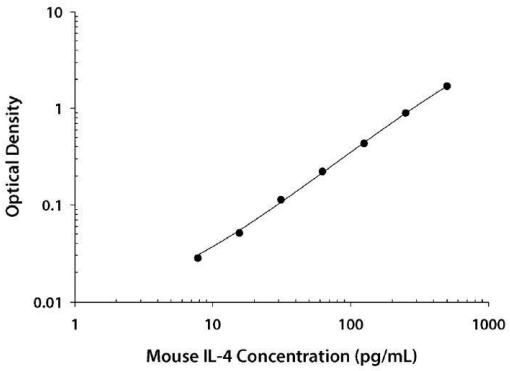
IL-2
IL-3
IL-4 I1
IL-5
IL-13

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 monoclonal antibody against mouse IL-4	1 plate
Mouse IL-4 Conjugate	Solution of a polyclonal 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×)	A 5× concentrated buffered protein base	1 vial
Wash Buffer concentrate (25×)	A 25× concentrated solution of buffered surfactant	1 vial
Color Reagent A	Stabilized hydrogen peroxide	1 vial
Color Reagent B	Stabilized chromogen (tetramethylbenzidine)	1 vial
Stop Solution	Diluted hydrochloric acid solution	1 vial
Plate Covers	Adhesive strip	3 strips

B. STORAGE

Unopened Kit	Store at 2-8°C. Do not use past kit expiration date.	
Opened/ Reconstituted Reagents	Diluted Wash Solution	May be stored for up to 1 month at 2-8°C.*
	Stop Solution	
	Diluent 1×	
	Conjugate	
	Unmixed Color Reagent A	
	Unmixed Color Reagent B	
	Standard	Aliquot and store for up to 1 month at -20°C in a manual defrost freezer.* Avoid repeated freeze-thaw cycles.
	Microplate Wells	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.*

* 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

- 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.

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.

B. SAMPLE PREPARATION

Serum samples require a 5-fold dilution. A suggested 5-fold dilution is 40 µL of sample + 160 µL of Diluent (1×).

C. REAGENT PREPARATION

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

Wash Solution - 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.

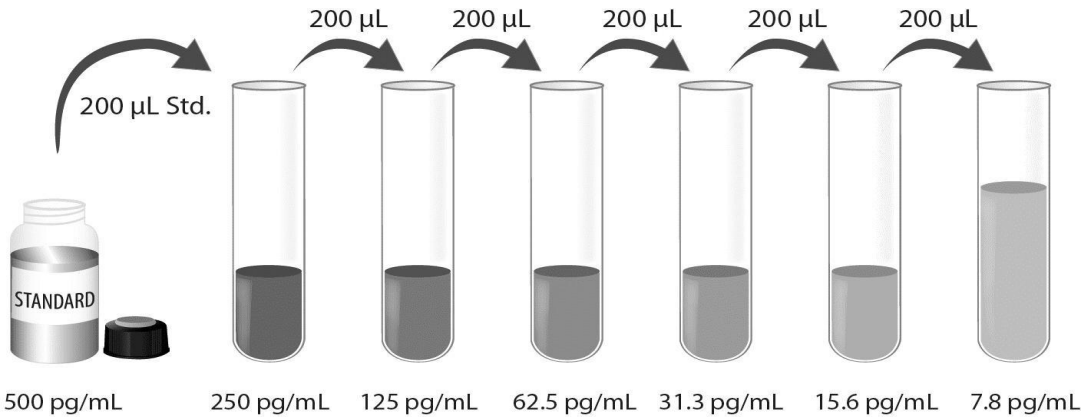
Substrate Solution - Color Reagent A and B should be mixed together in equal volumes within 15 minutes of use. Protect from light. 100 µL of the resultant mixture is required per well.

Diluent 1× - Add 20 mL of Calibrator Diluent 5× into 80 mL of deionized or distilled water to prepare 100 mL of 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 5 minutes with gentle agitation prior to making dilutions.

*if you have any question, please seek help from our Technical Support.

Pipette 200 µL of 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 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.
- 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 50 µL of Diluent 1× to each well.
4. Add 50 µL of Standard, 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.
5. 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.
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 Substrate Solution to each well. Incubate for 30 minutes at room temperature. **Protect from light**.
9. Add 100 µL of Stop Solution to each well. The color in the wells should change from blue to yellow. If the color in the wells is green or if the color change does not appear uniform, gently tap the plate to ensure thorough mixing.
10. Determine the optical density of each well within 30 minutes, using a microplate reader set to 450 nm. If wavelength correction is available, set to 540 nm or 570 nm. If wavelength correction is not available, subtract readings at 540 nm or 570 nm from the readings at 450 nm. This subtraction will correct for optical imperfections in the plate. Readings made directly at 450 nm without correction may be higher and less accurate.
11. **CALCULATION OF RESULTS.** Average the duplicate readings for each standard, control, and sample and subtract the average zero standard optical density. 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 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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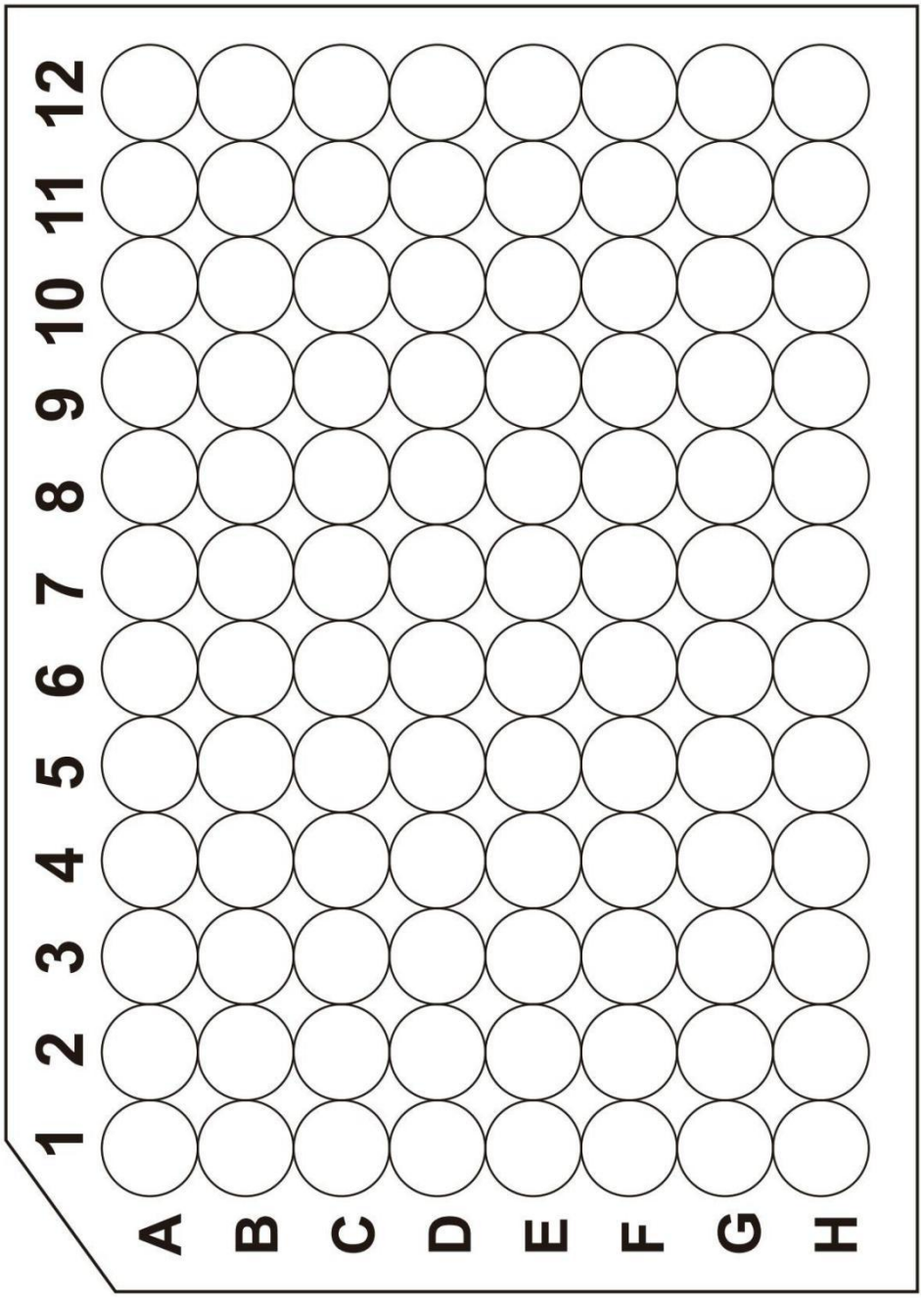
PLATE LAYOUT

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 的含量

科研专用，不可用于临床诊断

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Novus 试剂盒确保在你收货日期 3 个月内有效

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 18-20 kDa 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。不可理喻的是，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 单抗包被于微孔板上，样品和标准品中的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 多抗，与结合在微孔板上的 IL-4 结合而形成免疫复合物，游离的成分被洗去；加入底物溶液（显色剂），溶液颜色逐渐变成蓝色，加入终止液溶液变黄并且停止变化。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，应将样本用稀释剂（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、试剂盒的储存等。

C. 技术小提示

- 当混合或重溶蛋白液时，尽量避免起沫；
- 为了避免交叉污染，配置不同浓度标准品、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；
- 每次孵育时，正确使用封板胶纸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。

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	49.5	238	13.8
标准差	1.1	14.8	5.3	15.8	1.1
CV%	8.0	5.7	10.7	6.7	8.0

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75-112%，平均回收率在 90%。

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，然后用稀释剂（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 mM L-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。

血清样本 - 使用本试剂盒检测了 4 份鼠血清样本中 IL-4 的水平。4 份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。将以下因子用稀释剂（1×）配置成 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。

重组小鼠蛋白

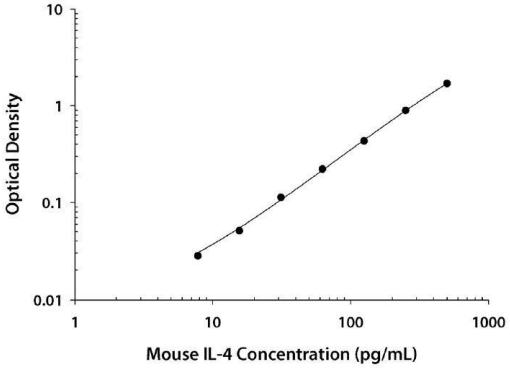
IL-2	IL-5
IL-3	IL-13
IL-4 I1	

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

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	包被抗体的 96 孔聚苯乙烯板，8 孔×12 条	1 块板
Mouse IL-4 Conjugate	酶标检测IL-4 抗体	1 瓶
Mouse IL-4 Standard	标准品（冻干）	1 瓶
Calibrator Diluent （5×）	浓缩稀释剂（5×）	1 瓶
Wash Buffer Concentrate（25×）	浓缩洗涤缓冲液（25×）	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 Covers	封板胶纸	3 张

* 本试剂盒包含足够的试剂以用于一块 96 孔微孔板的ELISA 实验。

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="8">已打开，稀释或重溶的试剂</td><td>洗涤缓冲液（1×）</td><td rowspan="6">2-8℃储存，最多 30 天*</td></tr>
<tr><td>终止液</td></tr>
<tr><td>稀释剂 1×</td></tr>
<tr><td>酶标检测抗体</td></tr>
<tr><td>显色剂 A</td></tr>
<tr><td>显色剂 B</td></tr>
<tr><td>标准品</td><td>分装，-20℃以下冰箱储存 30 天*；避免反复冻融</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

- 试剂盒中的终止液是酸性溶液，使用时请做好眼镜、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。样本可能需要用稀释剂（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。

B. 样本准备工作

血清样本需要用稀释剂（1×）5倍稀释后进行检测，即40 µL血清+160 µL稀释剂（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 20 mL 浓缩洗涤液用蒸馏水或去离子水稀释配置成 500 mL 工作浓度的洗涤液。未用完的放回 4℃。

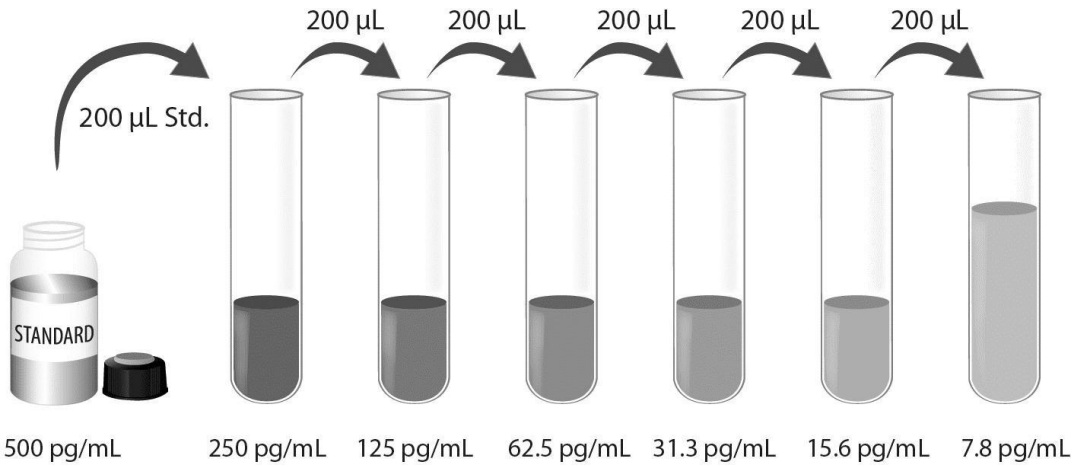
显色剂：按当次试验所需要用量将显色剂 A 和显色剂B 等体积混合，避光；在使用前 15 分钟内准备，仅供当日使用；每孔需 100 µL。

稀释剂（1×）：可将 20 mL 浓缩稀释剂用 80 mL 蒸馏水或去离子水稀释配置成 100 mL 工作浓度的稀释剂。

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 5 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 稀释剂（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），稀释剂（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，配置不同浓度标准品、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，正确使用封板胶纸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. 在每个微孔中加入 50 µL 稀释剂（1×）；
4. 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 50 µL。用封板胶纸封住反应孔，室温孵育 2 小时。说明书提供了一张 96 孔模板图，可用于记录标准品和试验样本的板内位置；
5. 将板内液体吸去，使用洗瓶、多通道洗板器或自动洗板机洗板。每孔加洗涤液 400 µL，然后将板内洗涤液吸去。重复操作 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。用封板胶纸封住反应孔，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 显色底物，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，孔内溶液颜色会从蓝色变为黄色。如果溶液颜色变为绿色或者颜色变化不一致，请轻拍微孔板，使溶液混合均匀；
10. 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 570nm 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；
11. **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。这个过程可生成一个足够使用但不太精确的数据拟合。若样本经过稀释，计算浓度时应乘以稀释倍数。

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