PRODUCT INFORMATION & MANUAL

Mouse IL-2 Valukine™ ELISA

Catalog Number: VAL602

For the quantitative determination of natural and recombinant mouse IL-2 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

IL-2 (also known as TCGF) is a 15-16 kDa monomeric, α-helical glycoprotein that belongs to the Type I cytokine family (1-3). Mouse IL-2 is synthesized as a 169 amino acid (aa) precursor that contains a 20 aa signal sequence and a 149 aa mature chain. There is also one potential site for O-linked glycosylation, and over residues 35-46 there is a poly-Gln region. Notably, IL-2 has multiple alleles in mouse, varying in the number of Gln residues and TSSS repeats over aa #21-46 in the sequence for Mus musculus (SwissProt #:P04351) (4). Mature mouse IL-2 shares 56% and 73% aa sequence identity with mature human and rat IL-2, respectively, with the major difference residing in the presence of multiple glutamines. Mouse and human IL-2 exhibit limited cross-species activity (4, 5).

Functionally, IL-2 was initially thought to be a growth factor for T cells, and as such, was tried therapeutically in humans in the hope that it might boost the immune response in cancer and HIV patients (6-9). Unfortunately, these efforts have shown limited success. Experimentally, studies have shown that mice lacking IL-2 or the IL-2R develop lymphoproliferation and autoimmune disease, suggesting that the function of IL-2 is more of a growth-limiting, rather than a growth-inducing factor (3, 6). Recent studies now suggest that the real reason for lymphoproliferation is an inability to produce CD4$^+$FoxP3$^+$CD25/IL-2Rα$^+$ Tregs (3). Notably, IL-2 is now suggested be an absolute requirement for successful CD8$^+$ memory cell recall responses (3). Mammalian cells known to express IL-2 include CD4$^+$ and CD8$^+$ T cells, NK and NKT cells, and dendritic cells (3). The receptor for the IL-2 is complex and consists of three distinct subunits in varying combinations (3, 10, 11). Two of these are ligand-binding and are termed IL-2Rα and IL-2Rβ. IL-2Rβ is 55 kDa and binds IL-2 with low affinity. IL-2Rβ, which is 75 kDa and is also a component of the IL-15 receptor, binds IL-2 with intermediate affinity. Signal transduction is performed by both IL-2Rβ and a 64 kDa common gamma chain (γc). The γc shares receptors with IL-4, -7, -9, -15, and -21. This signal transducing common gamma chain does not bind IL-2, but does heterodimerize with IL-2Rβ to form a functional IL-2 receptor. The complex heterotrimeric α-β-γc receptor may arise from IL-2 binding to preformed Rα-Rβ complexes (12).

II. OVERVIEW

A. PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for mouse IL-2 has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any mouse IL-2 present is bound by the immobilized antibody. After washing away any unbound substances, an enzyme-linked polyclonal antibody specific for mouse IL-2 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-2 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 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
Mean (pg/mL)	26.0	521	32.4	94.1	477
Standard Deviation	1.2	24.4	4.5	9.7	58.4
CV%	4.6	4.7	13.9	10.3	12.2

B. RECOVERY

The recovery of mouse IL-2 spiked to different levels throughout the range of the assay in cell culture media was evaluated. The recovery ranged from 84-107% with an average of 95%.

The recovery of mouse IL-2 spiked to different levels throughout the range of the assay in mouse serum was evaluated. The recovery ranged from 70.1-85.3% with an average of 80.5%.

C. SENSITIVITY

The minimum detectable dose (MDD) of mouse IL-2 is typically less than 3.5 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-2 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-2 and diluted with Diluent 1× to produce samples with values within the dynamic range of the assay.

Dilution	Average % of Expected	Range (%)
1:2	99	95 - 105
1:4	100	96 - 104
1:8	103	97 - 110
1:16	103	99 - 109

F. SAMPLE VALUES

Cell Culture Supernates - The following primary tissues from the mice were homogenized and seeded in RPMI supplemented with 10% fetal bovine serum, 2 mM L-glutamine, 100 U/mL penicillin, and 100 µg/mL streptomycin sulfate. The cell culture supernates were assayed for levels of mouse IL-2.

Primary Cell Type	# Organs homogenized	Seeded volume	Stimulant	Incubation Time	Detectable Levels (pg/mL)
Spleen	2	125 mL	N/A	2 days	ND
Spleen	2	100 mL	10µg/mL ConA	2 days	447

EL-4 cells (Mouse thymoma) were seeded at 2×10^5 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-2 and measured 19 ng/mL.

Serum - Four Mouse serum samples were evaluated for the presence of IL-2 in this assay. All samples measured below the lowest standard, 15.6pg/mL.

G. SPECIFICITY

This assay recognizes both natural and recombinant mouse IL-2. 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-2 control were assayed for interference. No significant cross-reactivity or interference was observed.

Recombinant mouse	
IL-4	IL-2 Rα
IL-7	IL-2 Rβ
IL-9	IL-2 Rγ
IL-15	

IV. EXPERIMENT

EXAMPLE STANDARD

The standard curve is provided for demonstration only. A standard curve should be generated for each set of samples assayed.
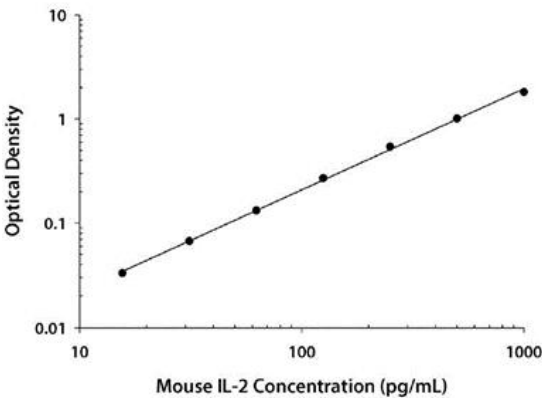
(pg/mL)	O.D.	Average	Corrected
0	0.050 0.050	0.050	—
15.6	0.083 0.083	0.083	0.033
31.3	0.117 0.117	0.117	0.067
62.5	0.187 0.178	0.182	0.132
125	0.321 0.319	0.320	0.270
250	0.576 0.610	0.593	0.543
500	1.056 1.064	1.060	1.010
1000	1.892 1.838	1.865	1.815

V. KIT COMPONENTS AND STORAGE

A. MATERIALS PROVIDED

Parts	Description	Size
Mouse IL-2 Microplate	96 well polystyrene microplate (12 strips of 8 wells) coated with monoclonal antibody against mouse IL-2	1 plate
Mouse IL-2 Conjugate	solution of a polyclonal antibody against mouse IL-2 conjugated to horseradish peroxidase	1 vial
Mouse IL-2 Standard	recombinant mouse IL-2 in a buffered protein base; lyophilized	2 vials
Calibrator Diluent(5×)	a 5×concentrated buffered protein base	1 vial
Wash Buffer concentrate (25×)	a 25× concentrated solution of buffered surfactant	1 vial
TMB Substrate	TMB ELISA Substrate Solution	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	
	Conjugate	
	TMB Substrate	
	Standard	Use a new standard for each assay.
	Calibrator Diluent Concentrate (5×)	May be stored for up to 1 month at 2-8 °C.* Use and discard diluted Calibrator Diluent (1×). Prepare fresh for each assay.
	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/buffer 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. REAGENT PREPARATION

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

C. REAGENT PREPARATION

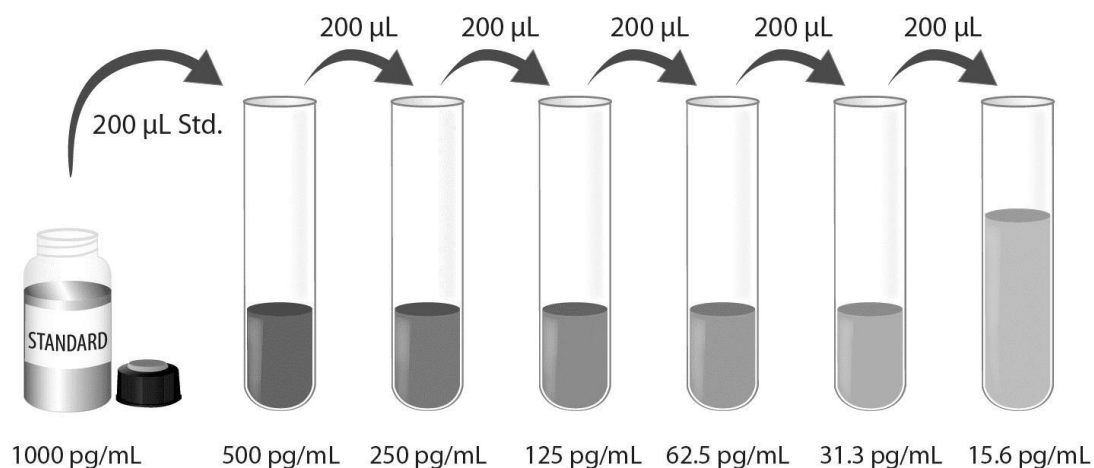*Note: Bring all reagents to room temperature before use.*

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.

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-2 Standard - Refer to the vial label for reconstitution volume*. This reconstitution produces a stock solution of 1000 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 1000 pg/mL serves as the high standard. The Diluent 1× serves as the zero standard (0 pg/mL).

200 µL Std.

200 µL   200 µL   200 µL   200 µL   200 µL

STANDARD

1000 pg/mL   500 pg/mL   250 pg/mL   125 pg/mL   62.5 pg/mL   31.3 pg/mL   15.6 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 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-2 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 IL-2 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-2 Valukine™ ELISA 试剂盒

目录号：VAL602

适用于定量检测天然和重组小鼠 IL-2 的含量

科研专用，不可用于临床诊断

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版本号 202307.4

目录

I. 背景

白细胞介素2 (IL-2,也称为TCGF）是一个15-16 kDa单体α螺旋糖蛋白，属于I型细胞因子家族（1-3）。小鼠IL-2前体是含169个氨基酸（aa）的多肽，它包含一个由20个氨基酸组成的信号序列和一个由149个氨基酸组成的成熟链。另外还有一个O-连接糖基化的潜在位点，以及在35-46残基处的多聚谷氨酰胺区。值得注意的是，小鼠IL-2有多个等位基因，导致小鼠的IL-2序列（SwissProt#:P04351）在21-46氨基酸处的谷氨酰胺数量及TSSS重复次数的不同（4）。成熟的小鼠IL-2氨基酸序列与人合大鼠的IL-2分别有56%和73%的同源性，主要差异位于聚谷氨酰胺区。小鼠和人的IL-2存在一定交叉性（4,5）。在功能上，IL-2最初被认为是T细胞生长因子，因此，被用于对癌症和艾滋病患者的治疗，以及提高免疫反应。然而，这些努力效果有限。实验研究显示，缺乏IL-2或IL-2受体的小鼠，也可产生淋巴细胞增生和患自身免疫性疾病，表明IL-2是一个限制增长，而不是诱导增长的因子（3,6）。最近的研究表明，淋巴细胞增生的真正原因是无法产生CD4$^+$FoxP3$^+$CD25/IL-2Rα$^+$ Treg细胞（3）。值得注意的是，现在IL-2被认为是成功诱导CD8$^+$记忆细胞回忆应答的必要条件（3）。总所周知，表达IL-2的哺乳动物细胞包括CD4$^+$和CD8$^+$T细胞、NK细胞、NKT细胞和树突状细胞（3）。IL-2受体很复杂，由三个不同亚基组合而成（3, 10, 11）。其中两个亚基可与配体结合，被称为IL-2Rα和IL-2Rβ。IL-2Rα为55 kDa，凭借低亲和力与IL-2结合。IL-2β为75 kDa，也是IL-15受体的组成部分，中等亲和力与IL-2结合。信号转导通过IL-2Rβ和一个64kDa的共同γ链（γc）来完成。γc与IL-4，-7，-9，-15和-21共享受体。信号转导γc不与IL-2结合，但与IL-2Rβ异源二聚体化，形成一个功能性IL-2受体。异源三聚体α-β-γc受体可能与IL-2结合，形成Rα-Rβ复合体（12）。

II. 概述

A. 检测原理

本实验采用双抗体夹心ELISA法。抗小鼠IL-2单抗包被于微孔板上，样品和标准品中的IL-2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-2多抗，未结合的抗体被洗去；加入TMB底物溶液，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。

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；

● 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。

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）	26	521	32.4	94.1	477
标准差	1.2	24.4	4.5	9.7	58.4
CV%	4.6	4.7	13.9	10.3	12.2

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-2，测定其回收率。回收率范围84-107%，平均回收率在95%。

在小鼠血清样本中掺入检测范围内不同水平的小鼠IL-2，测定其回收率。回收率范围70.1-85.3%，平均回收率在80.5%。

C. 灵敏度

小鼠IL-2的最低可测剂量（MDD）一般小于3.5pg/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-2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IL-2，然后用稀释液（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	99	95 - 105
1:4	100	96 - 104
1:8	103	97 - 110
1:16	103	99 - 109

F. 样本预值

细胞培养上清液 - 从小鼠脾脏组织匀浆得到原代细胞培养于RPMI培养基中，细胞培养基还含有10%胎牛血清、2mM L-谷氨酰胺、100U/mL青霉素和100μg/mL硫酸链霉素。取细胞培养上清液测定小鼠IL-2含量。

主要的细胞类型	用于匀浆的组织数量	培养的体积	激活剂	孵育时间	检测结果 (pg/mL)
脾	2	125 mL	N/A	2 days	ND
脾	2	100 mL	10 μg/mL ConA	2 days	447

EL-4细胞（小鼠胸腺瘤）以2×10^5 cells/mL铺板，培养4天；培养基为100mL的DMEM，含有10%马血清、10μg/mL PHA和10 ng/mL PMA。取上清液，测得IL-2的量为19ng/mL。

血清样本 - 使用本试剂盒检测了4份鼠血清样本中IL-2的水平。4份样本的检测值均低于最低标准品，15.6pg/mL。
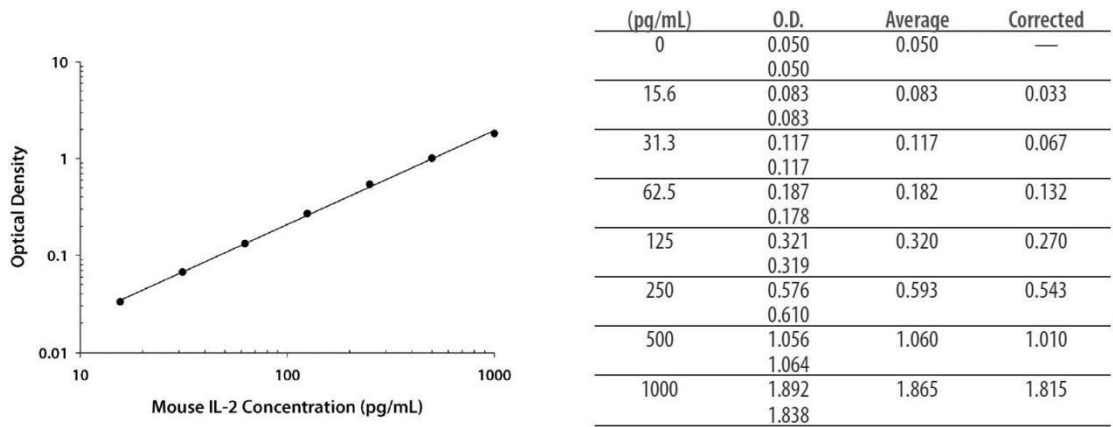
G. 特异性

此ELISA法可检测天然及重组小鼠IL-2蛋白。将以下因子用稀释液（1×）配制成50ng/mL的浓度来检测与小鼠IL-2的交叉反应。将50ng/mL的干扰因子掺入中间范围的重组小鼠IL-2对照品中，来检测对小鼠IL-2的干扰。没有观察到明显的交叉反应或干扰。

Recombinant mouse	
IL-4	IL-2 Rα
IL-7	IL-2 Rβ
IL-9	IL-2 Rγ
IL-15	

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	0.050	0.050	—
	0.050		
15.6	0.083	0.083	0.033
	0.083		
31.3	0.117	0.117	0.067
	0.117		
62.5	0.187	0.182	0.132
	0.178		
125	0.321	0.320	0.270
	0.319		
250	0.576	0.593	0.543
	0.610		
500	1.056	1.060	1.010
	1.064		
1000	1.892	1.865	1.815
	1.838		

V. 试剂盒组成及储存

A. 试剂盒组成

组成	描述	规格
Mouse IL-2 Microplate	包被抗体的96孔聚苯乙烯板，8孔 x 12条	1块板
Mouse IL-2 Conjugate	酶标检测IL-2抗体	1瓶
Mouse IL-2 Standard	标准品（冻干）	2瓶
Calibrator Diluent（5×）	浓缩稀释液（5 ×）	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 Covers	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	洗涤缓冲液（1×）	2-8℃储存，最多30天*
	终止液	
	酶标检测抗体	
	TMB底物溶液	
	标准品	已重溶的标准品仅限当天使用。
	标准品稀释液 (5×)	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,多余的丢弃
	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450nm检测波长的吸收值及540nm或570nm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

- 500mL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。

B. 样本准备工作

血清样本需要用稀释液（1×）2倍稀释后进行检测，即100 μL血清+100 μL稀释液（1×）。
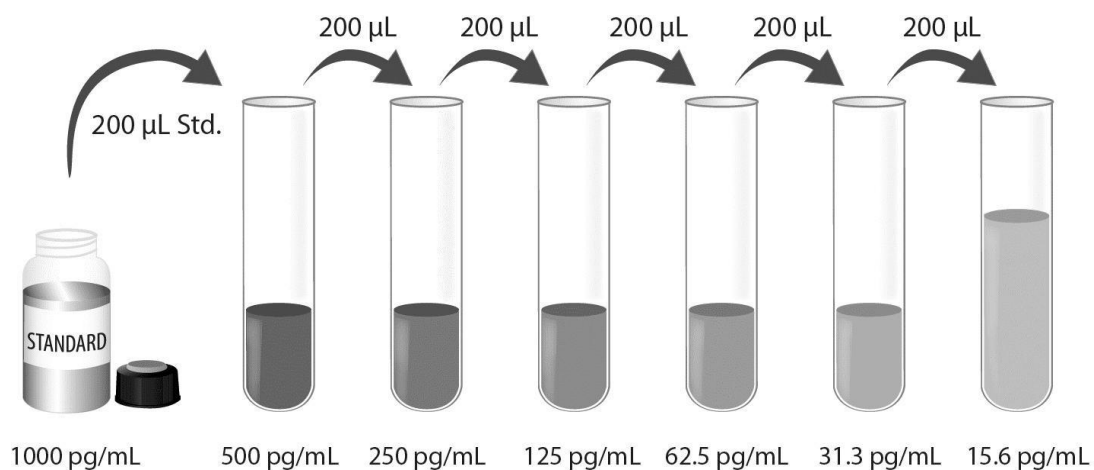
C. 检测前准备工作

使用前请将所有试剂放置于室温

洗涤液：从冰箱中取出的浓缩洗涤液可能有结晶，属于正常现象；放置室温，轻摇混匀，待结晶完全溶解后再配制洗涤液。可将20mL浓缩洗涤液用蒸馏水或去离子水稀释配制成500mL工作浓度的洗涤液。未用完的放回4℃。

稀释液（1×）：可将20mL浓缩稀释液用80mL蒸馏水或去离子水稀释配制成100mL工作浓度的稀释液。

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1000pg/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200μL稀释液（1×）。将标准品母液参照下图做系列稀释，每管需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（1000pg/mL），稀释液（1×）可用作标准曲线零点（0pg/mL）。

200 µL Std.   200 µL   200 µL   200 µL   200 µL   200 µL

1000 pg/mL   500 pg/mL   250 pg/mL   125 pg/mL   62.5 pg/mL   31.3 pg/mL   15.6 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. 在每微孔中加入50μL稀释液 (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50μL。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；

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400μL，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100μL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100μL 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100μL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-2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