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.

Bio-Techne China Co. Ltd
P: +86 (21) 52380373 P: 8009881270 F: +86 (21) 52381001
info.cn@bio-techne.com

Please refer to the kit label for expiry date.
Novus kits are guaranteed for 3 months from date of receipt

Version 202609.6

TABLE OF CONTENTS

I. BACKGROUND 2

II. OVERVIEW 3

III. ADVANTAGES 4

IV. EXPERIMENT 7

V. KIT COMPONENTS AND STORAGE 8

VI. PREPARATION 10

VII. ASSAY PROCEDURE 12

VIII. REFERENCES 14

I. BACKGROUND

Interleukin 2 (IL-2), also known as T cell growth factor (TCGF), is a 15-18 kDa variably glycosylated α-helical polypeptide that is a member of the common gamma chain (γc) cytokine family (1-4). It exists as a monomer and has a notably short half-life (< 30 minutes) (1). Mouse IL-2 is synthesized as a 169 amino acid (aa) precursor that contains a 20 aa signal sequence plus a 149 aa mature region (5, 6). The mature region is α-helical in nature and contains one utilized O-linked glycosylation site at Thr3, plus three cysteines, two of which form an intrachain disulfide bond that is essential for activity (7). Mature mouse IL-2 shares 56% and 73% aa identity with human and rat IL-2, respectively. Although human IL-2 shares only approximately 60% aa identity with the highly polymorphic mouse IL-2, human IL-2 is known to be active on mouse IL-2 responsive cells. Cells reported to secrete IL-2 include γδT cells (8), activated conventional $CD4^+$ and $CD8^+$ T cells (1, 9), neurons (10, 11), microglia (12), and hematopoietic stem cells (13).

The receptor for IL-2 (IL-2 R) is composed of three subunits, the 55 kDa CD25/IL-2 Rα chain, the 70 kDa IL-2 Rβ chain, and the 65 kDa γc (1, 3). IL-2 first binds to CD25; the binary complex then recruits IL-2 Rβ and γc to form the quaternary signaling complex (1, 14). In addition to IL-2, IL-2 Rβ is used by IL-15 in its quaternary signaling complex. γc also serves as a signaling receptor for IL-4, -7, -9, -15, and -21 (1, 3).

In vitro studies have shown an important role for IL-2 in T cell activation and expansion. In vivo, IL-2 is critical for the development, maintenance and function of regulatory T cells (Treg) which provide protection against autoimmune disease. On the other hand, IL-2 can also promote autoimmune inflammation in target organs through its roles in regulating the expression of T cell trafficking genes and production of Th2 cytokines. Within the $CD8^+$ T cell subset, IL-2 is essential for optimal primary responses and differentiation into terminal effector cells. IL-2 also promotes the development of activated $CD8^+$ T cells into memory cells (1).

II. OVERVIEW

A. PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. An 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 antibody specific for mouse IL-2 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-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 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 RD5T 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)	70.4	187	595	70.9	175	559
Standard Deviation	3.9	10.0	23.4	3.6	9.6	24.5
CV%	5.5	5.3	3.9	5.1	5.5	4.4

B. RECOVERY

The recovery of mouse IL-2 spiked to three levels throughout the range of the assay was evaluated.

Sample Type	**Average % Recovery**	**Range (%)**
Cell culture supernates (n=6)	102	84-110
Serum (n=6)	102	96-108

C. SENSITIVITY

The minimum detectable dose (MDD) of mouse IL-2 is typically less than 3.0 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.

The NIBSC non-WHO reference mouse IL-2 preparation 93/566, which was intended as a bioassay standard, was evaluated in this kit. Each ampoule contained a nominal 0.1 µg of recombinant mouse IL-2 and was assigned an arbitrary unitage of 10,000 U/ampoule. NIBSC 93/566: 1 Unit of Standard = 5.3 pg of Valukine Mouse IL-2

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 Calibrator Diluent RD5T to produce samples with values within the dynamic range of the assay.

		Cell culture supernates (n=8)	**Serum (n=8)**
1:2	Average % of Expected	99	102
	Range (%)	97-103	98-107
1:4	Average % of Expected	97	104
	Range (%)	91-105	93-112
1:8	Average % of Expected	94	99
	Range (%)	87-101	89-107
1:16	Average % of Expected	92	94
	Range (%)	83-103	82-108

F. SAMPLE VALUES

Serum - Forty samples were evaluated for detectable levels of mouse IL-2 in this assay. Thirty-nine measured less than the lowest mouse IL-2 standard, 15.6 pg/mL. One sample read 48 pg/mL.

Cell Culture Supernates - EL-4 mouse lymphoblast cells (10^5 cells/mL) were cultured for 2 days in DMEM supplemented with 10% fetal bovine serum and stimulated with 10 µg/mL PHA and 10 ng/mL PMA. The culture supernate was assayed for mouse IL-2 and measured 40 ng/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.

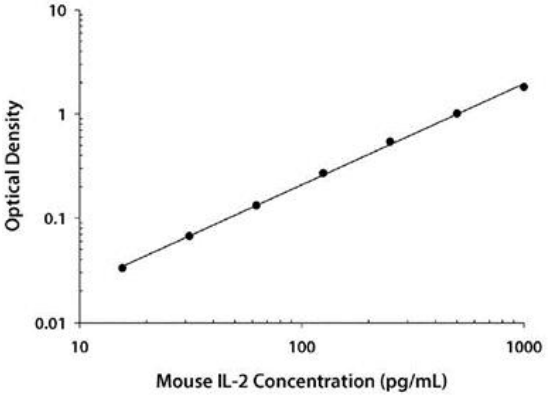
Recombinant mouse:		**Recombinant human:**
C10	IL-13	IL-2
G-CSF	JE/MCP-1	IL-2 Rα
GM-CSF	KC	IL-2 Rβ
IFN-γ	LIF	**Recombinant porcine:**
IL-1α	M-CSF	IL-2
IL-1β	MIP-1α	
IL-3	MIP-1β	
IL-4	MIP-2	
IL-5	SCF	
IL-6	TNF-α	
IL-7	Tpo	
IL-9	VEGF	

A sample containing 50 ng/mL of recombinant rat IL-2 read 250 pg/mL in this assay (0.5% cross-reactivity). Upon dilution, the dose-curve of recombinant rat IL-2 was parallel to the mouse IL-2 standard curve.

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 an antibody against mouse IL-2.	1 plate
Mouse IL-2 Conjugate	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
Assay Diluent RD1-14	A buffered protein solution with preservatives. *May contain a precipitate. Mix well before and during use.*	1 vial
Calibrator Diluent RD5T	A 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="8">Opened/ Reconstituted Reagents</td><td>Wash Buffer (1×)</td><td rowspan="5">May be stored for up to 1 month at 2-8°C.*</td></tr>
<tr><td>Assay Diluent RD1-14</td></tr>
<tr><td>Calibrator Diluent RD5T</td></tr>
<tr><td>Stop Solution</td></tr>
<tr><td>Conjugate</td></tr>
<tr><td>TMB Substrate</td><td rowspan="2">Use a new standard for each assay.
Discard after use.</td></tr>
<tr><td>Standard</td></tr>
<tr><td>Microplate Wells</td><td>Return unused wells to the foil pouch containing the desiccant pack, reseal along entire edge of zip-seal. May be stored for up to 1 month at 2-8°C.*</td></tr>
</table>

* Provided this is within the expiration date of the kit.

C. OTHER SUPPLIES REQUIRED

- Microplate reader capable of measuring absorbance at 450 nm, with the correction wavelength set at 540 nm or 570 nm.
- Pipettes and pipette tips.
- Deionized or distilled water.
- Squirt bottle, manifold dispenser, or automated microplate washer.
- 500 mL graduated cylinder.

D. PRECAUTION

- The Stop Solution provided with this kit is an acid solution.
- Some components in this kit contain a preservative which may cause an allergic skin reaction. Avoid breathing mist.
- Wear protective gloves, clothing, eye, and face protection. Wash hands thoroughly after handling.

VI. PREPARATION

A. SAMPLE COLLECTION AND STORAGE

Use polypropylene tubes.

The sample collection and storage conditions listed below are intended as general guidelines. Sample stability has not been evaluated.

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 RD5T.

Serum - Allow blood samples to clot for 2 hours at room temperature before centrifuging for 20 minutes at 2000 × 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 RD5T.

Note: *Grossly hemolyzed or lipemic samples may not be suitable for use in this assay.*

B. REAGENT PREPARATION

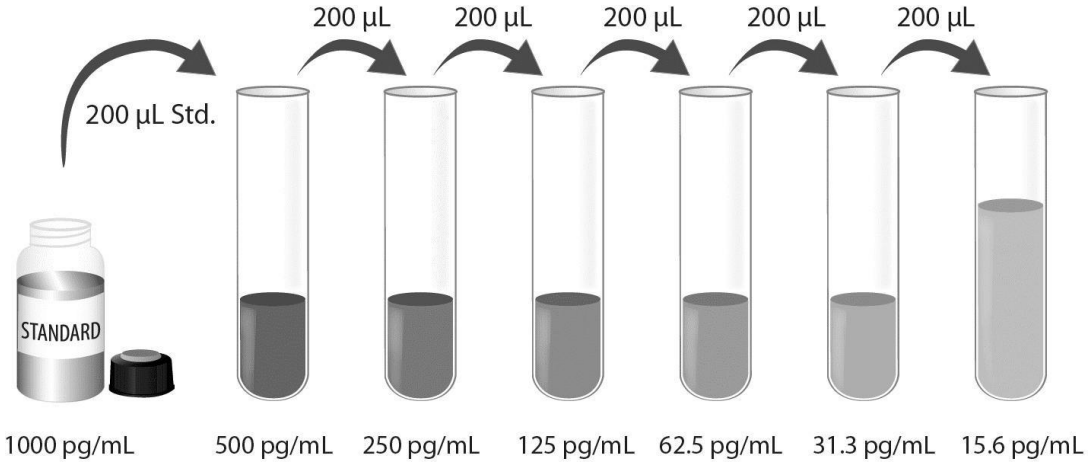
Note: *Bring all reagents to room temperature before use.*

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×).

Mouse IL-2 Standard - Refer to the vial label for reconstitution volume*. Reconstitute the Mouse IL-2 Standard with Calibrator Diluent RD5T. Do not substitute other diluents. 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.

Use polypropylene tubes. Pipette 200 µL of Calibrator Diluent RD5T 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 Calibrator Diluent RD5T serves as the zero standard (0 pg/mL).



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.
- 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 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 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 Assay Diluent RD1-14 to each well. *RD1-14 may contain undissolved material even when mixed well before and during its use.*
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 four times for a total of five 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-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 mouse 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.

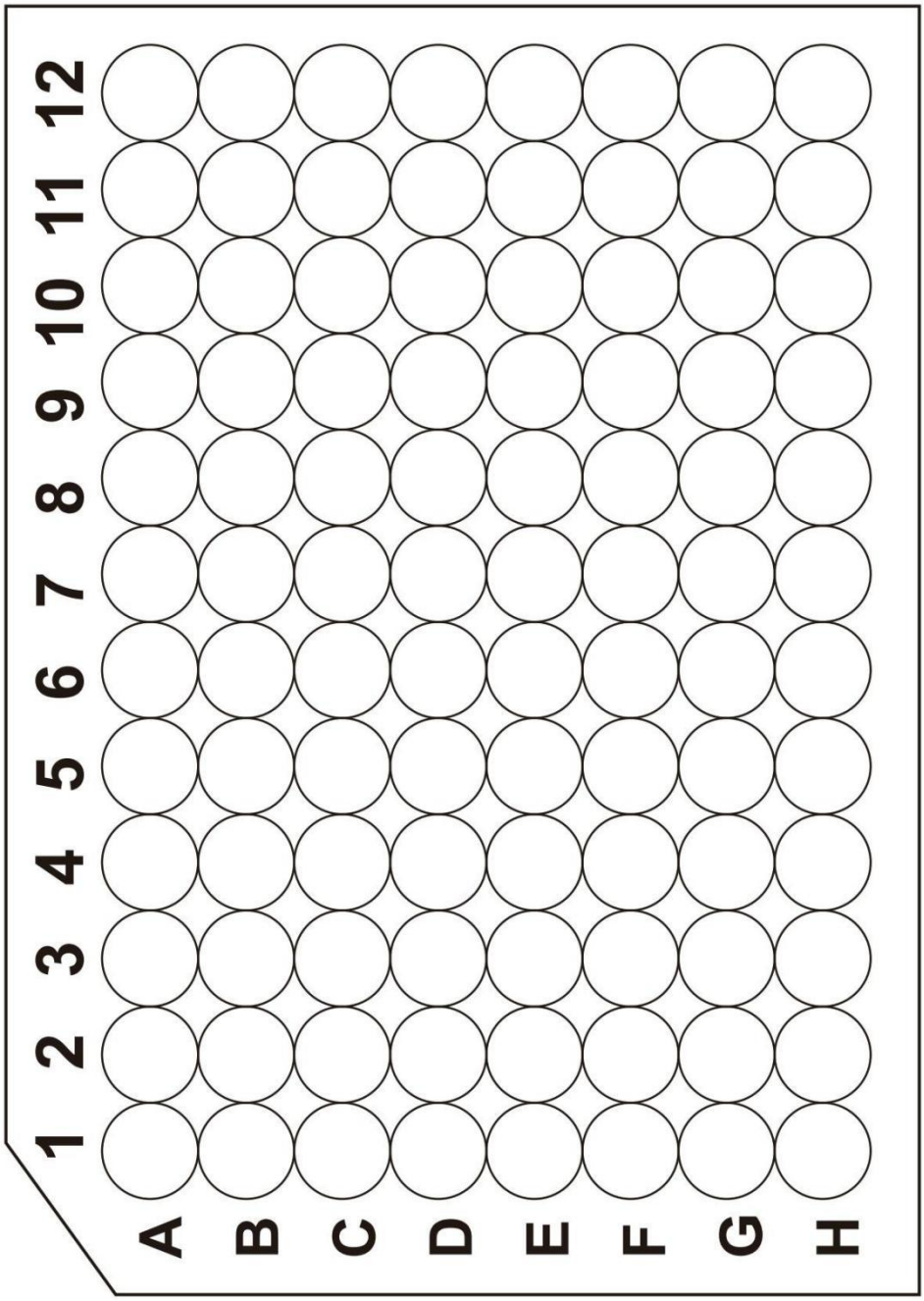
If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.

VIII. REFERENCES

1. Malek, T.R and I. Castro (2010) Immunity 33:153.
2. Waldman, T.A. (2006) Nat. Rev. Immunol. 6:595.
3. Malek, T.R. (2008) Annu. Rev. Immunol. 26:453.
4. Conradt, H.S. et al. (1989) J. Biol. Chem. 264:17368.
5. Kashima, N. et al. (1985) Nature 313:402.
6. Rosenberg, S.A. et al. (1984) Science 223:1412.
7. Smith, K.A. (1984) Annu. Rev. Immunol. 2:319.
8. Tsukaguchi, K. et al. (1995) J. Immunol. 154:1786.
9. Conlon, K. et al. (1995) Eur. J. Immunol. 25:644.
10. Hwang, I.K. et al. (2006) Brain Res. 1106:197.
11. Giestal de Araujo, E. et al. (2009) Ann. N.Y. Acad. Sci. 1153:57.
12. Kowalski, J. et al. (2004) Pol. J. Pharmacol. 56:563.
13. Eguizabal, C. et al. (2007) Int. J. Dev. Biol. 51:731.
14. Stauber, D.J. et al. (2006) Proc. Natl. Acad. Sci. USA 103:2788.

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 的浓度

科研专用，不可用于临床诊断

Bio-Techne China Co. Ltd

P: +86 (21) 52380373 P: 8009881270 F: +86 (21) 52381001

info.cn@bio-techne.com

有效期详见试剂盒包装标签

Novus 试剂盒确保在你收货日期 3 个月内有效

版本号 202609.6

目录

I. 背景 18

II. 概述 19

III. 优势 20

IV. 实验 23

V. 试剂盒组成及储存 24

VI. 实验前准备 26

VII. 操作步骤 28

VIII. 参考文献 29

I. 背景

白介素2（IL-2），又称T细胞生长因子（TCGF），是一种15-18 kDa的可变糖基化α-螺旋多肽，属于共同γ链（γc）细胞因子家族成员（1-4）。它以单体形式存在，半衰期显著较短（< 30分钟）（1）。小鼠IL-2以169个氨基酸（aa）的前体形式合成，该前体包含一个20个氨基酸的信号序列和一个149个氨基酸的成熟区域（5, 6）。成熟区域本质上是α-螺旋结构，在Thr3处含有一个被利用的O-连接糖基化位点，以及三个半胱氨酸，其中两个形成对活性至关重要的链内二硫键（7）。成熟小鼠IL-2与人IL-2和大鼠IL-2的氨基酸同一性分别为56%和73%。尽管人IL-2与高度多态的小鼠IL-2仅有约60%的氨基酸同一性，但已知人IL-2对小鼠IL-2反应性细胞具有活性。据报道分泌IL-2的细胞包括γδT细胞（8）、活化的常规$CD4^+$和$CD8^+$ T细胞（1, 9）、神经元（10, 11）、小胶质细胞（12）和造血干细胞（13）。

IL-2的受体（IL-2 R）由三个亚基组成：55 kDa的CD25/IL-2 Rα链、70 kDa的IL-2 Rβ链和65 kDa的γc（1, 3）。IL-2首先与CD25结合；二元复合物随后招募IL-2 Rβ和γc，形成四元信号复合物（1, 14）。除IL-2外，IL-2 Rβ还被IL-15用于其四元信号复合物。γc还作为IL-4、-7、-9、-15和-21的信号受体（1, 3）。

体外研究已表明IL-2在T细胞活化和扩增中发挥重要作用。在体内，IL-2对调节性T细胞（Treg）的发育、维持和功能至关重要，而Treg提供针对自身免疫性疾病的保护。另一方面，IL-2也可通过调节T细胞运输基因的表达和Th2细胞因子的产生，在靶器官中促进自身免疫性炎症。在$CD8^+$T细胞亚群中，IL-2对最佳初次应答和向终末效应细胞的分化至关重要。IL-2还促进活化的$CD8^+$ T细胞发育为记忆细胞（1）。

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抗体包被于微孔板上，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RD5T稀释后重新检测；
- 检测结果的不同可由多种因素引起，包括实验人员的操作、移液器的使用方式、洗板技术、反应时间或温度、试剂盒的效期等。

III. 优势

A. 精确度

板内精确度（同一板内不同孔间的精确度）

已知浓度的三个样本，在同一板内分别检测20次，以确定板内精确度。

板间精确度（不同板之间的精确度）

已知浓度的三个样本，在不同板中分别检测20次，以确定板间精确度。

	板内精确度			板间精确度		
样本	1	2	3	1	2	3
平均值 (pg/mL)	70.4	187	595	70.9	175	559
标准差	3.9	10.0	23.4	3.6	9.6	24.5
CV%	5.5	5.3	3.9	5.1	5.5	4.4

B. 回收率

对小鼠IL-2在整个检测范围内加标至三个水平的回收率进行了评估。

样本类型	平均回收率 (%)	范围 (%)
细胞培养上清 (n=6)	102	84-110
血清 (n=6)	102	96-108

C. 灵敏度

小鼠IL-2的最低可测剂量（MDD）一般小于3.0 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-2蛋白所校正。

NIBSC非WHO参考品小鼠IL-2制剂93/566，原本用作生物活性测定标准品，已在本试剂盒中进行了评估。每安瓿名义上含有0.1 µg重组小鼠IL-2，并被赋予10,000 U/安瓿的任意单位值。NIBSC 93/566：1单位标准品 = 5.3 pg Valukine小鼠IL-2。

E. 线性

不同的样本中含有或掺入高浓度的小鼠IL-2，然后用标准品稀释液RD5T将样本稀释到检测范围内，测定其线性。

		细胞培养上清 (n=8)	血清 (n=8)
1:2	平均值/期待值（%）	99	102
	范围 (%)	97-103	98-107
1:4	平均值/期待值（%）	97	104
	范围 (%)	91-105	93-112
1:8	平均值/期待值（%）	94	99
	范围 (%)	87-101	89-107
1:16	平均值/期待值（%）	92	94
	范围 (%)	83-103	82-108

F. 样本预值

小鼠血清样本 - 在本检测中，对四十份样本进行了小鼠IL-2可检测水平的评估。其中三十九份的测定值低于最低小鼠IL-2标准品浓度，即15.6 pg/mL。一份样本读数为48 pg/mL。

细胞培养上清- EL-4小鼠淋巴母细胞（10^5个细胞/mL）在补充有10%胎牛血清的DMEM中培养2天，并用10 µg/mL PHA和10 ng/mL PMA刺激。对培养上清进行小鼠IL-2检测，测定值为40 ng/mL。

G. 特异性

此ELISA法可检测天然及重组小鼠IL-2蛋白。

将以下因子配制成50 ng/mL的浓度来检测与小鼠IL-2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-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:		**Recombinant human:**
C10	IL-13	IL-2
G-CSF	JE/MCP-1	IL-2 Rα
GM-CSF	KC	IL-2 Rβ
IFN-γ	LIF	**Recombinant porcine:**
IL-1α	M-CSF	IL-2
IL-1β	MIP-1α	
IL-3	MIP-1β	
IL-4	MIP-2	
IL-5	SCF	
IL-6	TNF-α	
IL-7	Tpo	
IL-9	VEGF	

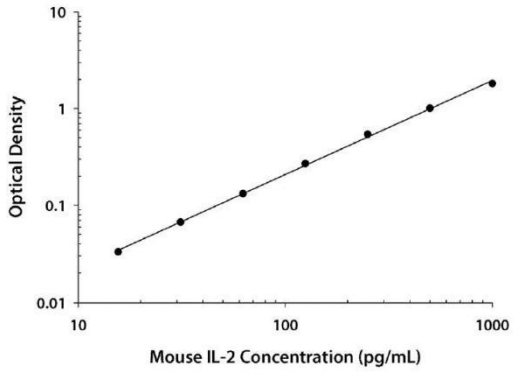
含有50 ng/mL重组大鼠IL-2的样本在本检测中读数为250 pg/mL（交叉反应性为0.5%）。

经稀释后，重组大鼠IL-2的剂量曲线与小鼠IL-2标准曲线平行。

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	0.083	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. 试剂盒组成及储存

A. 试剂盒组成

组成	描述	规格
Mouse IL-2 Microplate	包被抗小鼠IL-2抗体的96孔聚苯乙烯板，8孔 x 12条	1块板
Mouse IL-2 Conjugate	酶标检测抗小鼠IL-2抗体	1瓶
Mouse IL-2 Standard	小鼠IL-2标准品（冻干），参考瓶身标签进行重溶	2瓶
Assay Diluent RD1-14	检测液RD1-14	1瓶
Calibrator Diluent RD5T	标准品稀释液RD5T，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="7">已打开，稀释或重溶的试剂</td><td>洗涤液（1×）</td><td rowspan="5">2-8℃储存，最多30天*</td></tr>
<tr><td>检测液RD1-14</td></tr>
<tr><td>标准品稀释液 RD5T</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><td rowspan="2">每次检测使用新的标准品。使用后丢弃。</td></tr>
<tr><td>标准品</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

- 本试剂盒提供的终止液为酸性溶液。
- 本试剂盒中某些组分含有防腐剂，可能引起过敏性皮肤反应。避免吸入雾气。
- 穿戴防护手套、防护服、护目镜和面罩。操作后彻底洗手

VI. 实验前准备

A. 样品收集及储存

使用聚丙烯管。

以下列出的样本采集和储存条件仅为一般性指南。样本稳定性尚未经过评估。

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RD5T稀释。

血清样本：将血液样本在室温下凝固2小时，然后以2000 × g离心20分钟。取出血清并立即检测，或分装后于≤ -20° C储存。避免反复冻融。样本可能需要用标准品稀释液RD5T进行稀释。

注意：*严重溶血或脂血的样本可能不适合用于本检测。*

B. 检测前准备工作

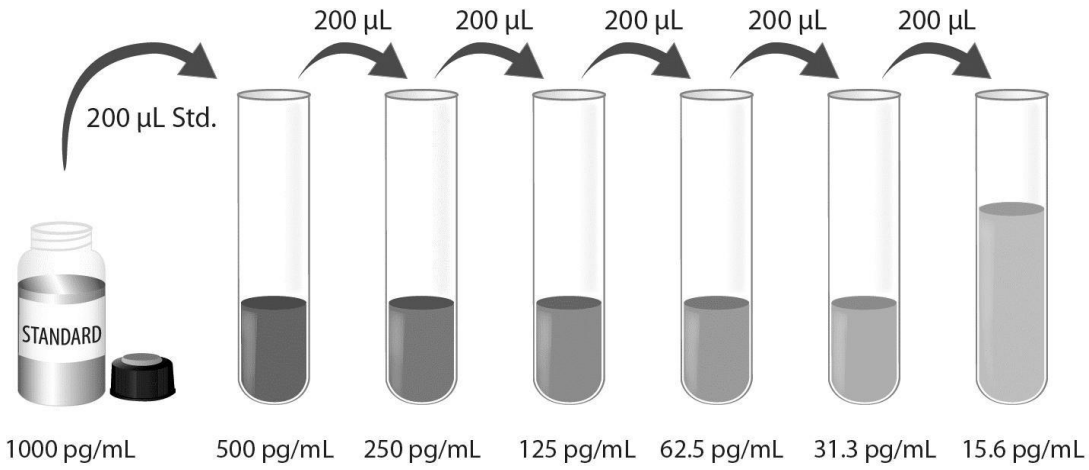
使用前请将所有试剂放置于室温

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×）。

小鼠IL-2标准品：参照标准品瓶身注明的方式重溶冻干标准品*。用标准品稀释液RD5T复溶小鼠IL-2标准品。请勿替换为其他稀释液。得到浓度为1000 pg/mL标准品母液。轻轻震摇至少5分钟，使其充分溶解。

*如有疑问，请咨询我们的技术支持。

使用聚丙烯管。每个稀释管中加入200 µL标准品稀释液RD5T。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（1000 pg/mL），标准品稀释液RD5T可用作标准曲线零点（0 pg/mL）。



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；
- 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. 每孔加入50 µL检测稀释液RD1-14。*RD1-14即使在使用前和使用过程中充分混匀，也可能含有未溶解的物质。*
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4次，共洗5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-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

1. Malek, T.R and I. Castro (2010) Immunity 33:153.
2. Waldman, T.A. (2006) Nat. Rev. Immunol. 6:595.
3. Malek, T.R. (2008) Annu. Rev. Immunol. 26:453.
4. Conradt, H.S. et al. (1989) J. Biol. Chem. 264:17368.
5. Kashima, N. et al. (1985) Nature 313:402.
6. Rosenberg, S.A. et al. (1984) Science 223:1412.
7. Smith, K.A. (1984) Annu. Rev. Immunol. 2:319.
8. Tsukaguchi, K. et al. (1995) J. Immunol. 154:1786.
9. Conlon, K. et al. (1995) Eur. J. Immunol. 25:644.
10. Hwang, I.K. et al. (2006) Brain Res. 1106:197.
11. Giestal de Araujo, E. et al. (2009) Ann. N.Y. Acad. Sci. 1153:57.
12. Kowalski, J. et al. (2004) Pol. J. Pharmacol. 56:563.
13. Eguizabal, C. et al. (2007) Int. J. Dev. Biol. 51:731.
14. Stauber, D.J. et al. (2006) Proc. Natl. Acad. Sci. USA 103:2788.

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

