PRODUCT INFORMATION & MANUAL

Rat IL-2 Valukine™ ELISA

Catalog Number: VAL907

For the quantitative determination of natural and recombinant rat Interleukin 2 (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 202506.1

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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 (γ_c) cytokine family (1-4). It exists as a monomer and has a notably short half-life (< 30 minutes) (1). Rat IL-2 is synthesized as a 155 amino acid (aa) precursor that contains a 20 aa signal sequence plus a 135 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 rat IL-2 shares 66% and 73% aa identity with human and mouse 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 γδ T 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 γ_c (1, 3). IL-2 first binds to CD25; the binary complex then recruits IL-2 Rβ and γ_c 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. The 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 rat IL-2 has been pre-coated onto a microplate. Standards, control and samples are pipetted into the wells and any rat IL-2 present is bound by the immobilized antibody. After washing away any unbound substances, an enzyme-linked antibody specific for rat IL-2 is added to the wells. Following a wash to remove any unbound antibody-enzyme reagent, TMB substrate (Chromogenic agent) is added to the wells and color develops in proportion to the amount of rat 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 supernates, rat plasma and rat 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 RD5-4 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)	130	478	1491	139	495	1550
Standard Deviation	4.2	10.5	31.9	13.8	30.8	85.4
CV%	3.2	2.2	2.1	9.9	6.2	5.5

B. RECOVERY

The recovery of rat IL-2 spiked to three levels throughout the range of the assay in various matrices was evaluated.

Sample Type	Average % Recovery	Range (%)
Cell culture supernates (n=8)	110	93-120
Rat serum* (n=14)	96	83-114
Rat EDTA plasma* (n=4)	102	90-117
Rat heparin plasma* (n=4)	100	92-116

*Samples were diluted prior to assay as directed in the Sample Preparation section.

C. SENSITIVITY

The minimum detectable dose (MDD) of rat IL-2 is typically less than 15 pg/mL.

The MDD was determined by adding two standard deviations to the mean optical density value of twenty zero standard replicates and calculating the corresponding concentration.

D. CALIBRATION

This immunoassay is calibrated against a highly purified *E. coli*-expressed recombinant rat IL-2 produced at R&D Systems.

E. LINEARITY

To assess the linearity of the assay, four or more samples containing and/or spiked with concentrations of rat IL-2 were diluted with Calibrator Diluent RD5-4 and then assayed.

Samples	Dilution	Observed (pg/mL)	Expected (pg/mL)	Observed / Expected x 100
Cell culture supernates	Neat	1627	____	____
	1:2	851	814	105%
	1:4	413	407	101%
	1:8	210	204	103%
	1:16	101	102	99%
Rat serum*	Spiked	1057	____	____
	1:2	557	528	105%
	1:4	286	264	108%
	1:8	132	132	100%
	1:16	65	66	98%
Rat EDTA plasma*	Spiked	876	____	____
	1:2	462	438	106%
	1:4	238	219	109%
	1:8	127	110	115%
	1:16	64	55	116%
Rat heparin plasma*	Spiked	1023	____	____
	1:2	561	512	110%
	1:4	279	256	109%
	1:8	131	128	102%
	1:16	68	64	106%

* Samples were spiked and then diluted prior to assay as directed in the Sample Preparation section.

F. SAMPLE VALUES

Rat serum - Forty serum samples were evaluated for the presence of rat IL-2 in this assay. Thirty-nine samples measured below the lowest standard, 31.3 pg/mL. One sample measured 238 pg/mL.

Rat plasma - Twenty EDTA plasma samples and fourteen heparin plasma samples were evaluated for the presence of rat IL-2 in this assay. Most of the samples measured below the lowest standard, 31.3 pg/mL. Two EDTA plasma samples measured 80 pg/mL and 92 pg/mL, respectively. One heparin plasma sample measured 62 pg/mL.

Cell Culture Supernates - Rat splenocytes (1 x 10^7 cells/mL) were cultured for 3 days in RPMI plus 10% fetal bovine serum and stimulated with 5.0 µg/mL Concanavalin A. An aliquot of the cell culture supernate was removed, assayed for rat IL-2, and measured 14 ng/mL.

G. SPECIFICITY

This assay recognizes natural and recombinant rat IL-2.

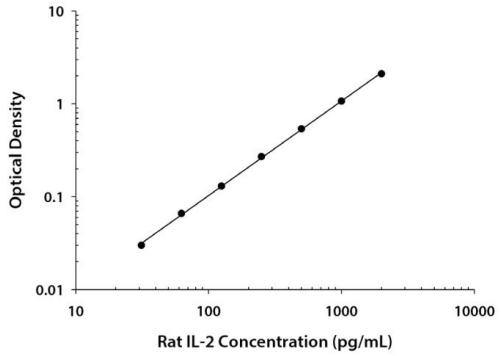
The factors listed below were prepared at 50 ng/mL in Calibrator Diluent RD5-4 and assayed for cross-reactivity. Preparations of the following factors at 50 ng/mL in a mid-range rat IL-2 control were assayed for interference. No significant cross-reactivity or interference was observed.

Recombinant rat:		**Recombinant mouse:**
CINC-1	IL-4	IL-2
GDNF	β-NGF	**Recombinant human:**
IFN-γ	PDGF-BB	IL-2
IL-1α	TNF-α	IL-2 Rα
		IL-2 Rβ

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	0.034 0.034	0.034	—
31.3	0.065 0.063	0.064	0.030
62.5	0.105 0.096	0.100	0.066
125	0.166 0.163	0.164	0.130
250	0.308 0.300	0.304	0.270
500	0.574 0.571	0.572	0.538
1000	1.118 1.087	1.102	1.068
2000	2.161 2.117	2.139	2.105

V. KIT COMPONENTS AND STORAGE

A. MATERIALS PROVIDED

Parts	Description	Size
Rat IL-2 Microplate	96 well polystyrene microplate (12 strips of 8 wells) coated with an antibody against rat IL-2.	1 plate
Rat IL-2 Conjugate	An antibody specific for rat IL-2 conjugated to horseradish peroxidase.	1 vial
Rat IL-2 Standard	Recombinant rat IL-2 in a buffered protein base; lyophilized. Refer to the vial label for reconstitution volume.	1 vial
Rat IL-2 Control	Recombinant rat IL-2 in a buffered protein base; lyophilized. The assay value of the control should be within the range specified on the vial label.	1 vial
Assay Diluent RD1-21	A buffered protein base.	1 vial
Calibrator Diluent RD5-4	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.	2 vials
Stop Solution	Diluted hydrochloric acid.	1 vial
Plate Sealers	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="6">May be stored for up to 1 month at 2-8°C.*</td></tr>
<tr><td>Assay Diluent RD1-21</td></tr>
<tr><td>Stop Solution</td></tr>
<tr><td>Conjugate</td></tr>
<tr><td>TMB Substrate</td></tr>
<tr><td>Calibrator Diluent RD5-4</td></tr>
<tr><td>Control</td><td rowspan="2">Use a new standard and control 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.
- Test tubes for dilution of standards and samples.

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

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 RD5-4.

Serum - Allow blood samples to clot for 2 hours at room temperature before centrifuging for 20 minutes at 1000 x 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 RD5-4.

Plasma - Collect plasma using EDTA or heparin as an anticoagulant. Centrifuge for 20 minutes at 1000 x g within 30 minutes of collection. Assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles. Samples may require dilution with Calibrator Diluent RD5-4.

Note: *Citrate plasma has not been validated for use in this assay*.

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

B. SAMPLE PREPARATION

Rat serum and plasma samples recommend a 2-fold dilution prior to assay. A suggested 2-fold dilution is 75 µL of sample + 75 µL of Calibrator Diluent RD5-4. Optimal dilutions should be determined by the end user.

C. REAGENT PREPARATION

Bring all reagents to room temperature before use.

Rat IL-2 Control - Reconstitute the control with 1.0 mL of deionized or distilled water. Mix thoroughly. Assay the control undiluted.

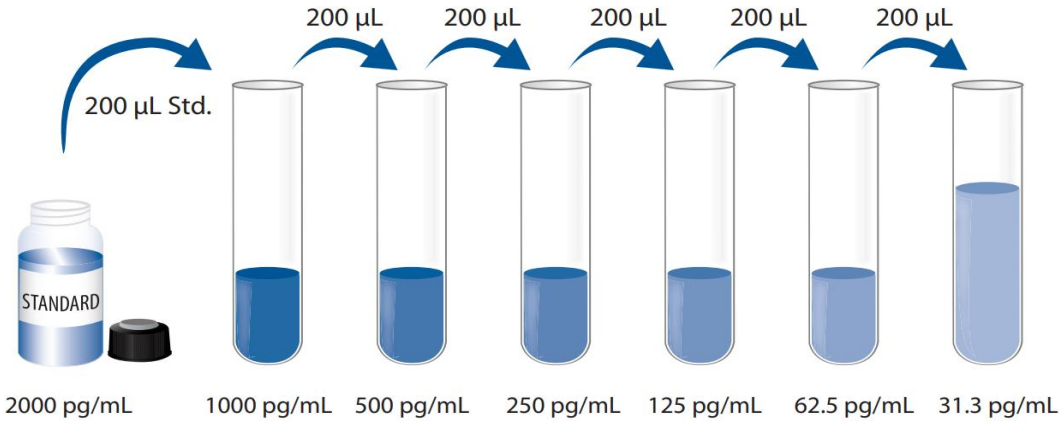
Wash Buffer (1×) - If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. Dilute 20 mL of Wash Buffer Concentrate (25×) into deionized or distilled water to prepare 500 mL of Wash Buffer (1×).

Rat IL-2 Standard- Refer to the vial label for the reconstitution volume*

Reconstitute the Rat IL-2 Standard with Calibrator Diluent RD5-4. Do not substitute other diluents. This reconstitution produces a stock solution of 2000 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 RD5-4 into each tube. Use the standard stock solution to produce a dilution series (below). Mix each tube thoroughly before the next transfer. The undiluted Rat IL-2 Standard (2000 pg/mL) serves as the high standard. The Calibrator Diluent RD5-4 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

Bring all reagents and samples to room temperature before use. It is recommended that all samples, controls and standards be assayed in duplicate.

1. Prepare all reagents, standards, control and samples 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 RD1-21 to each well.
4. Add 50 µL of standard, control and prepared sample per well. Mix by gently tapping the plate frame for 1 minute. 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 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 Rat 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, control and sample and subtract the average zero standard optical density (O.D.). Create a standard curve by reducing the data using computer software capable of generating a log/log 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 a log/log graph. The data may be linearized by plotting the log of the Rat IL-2 concentrations versus the log of the O.D. on a linear scale and the best fit line can be determined by regression analysis.

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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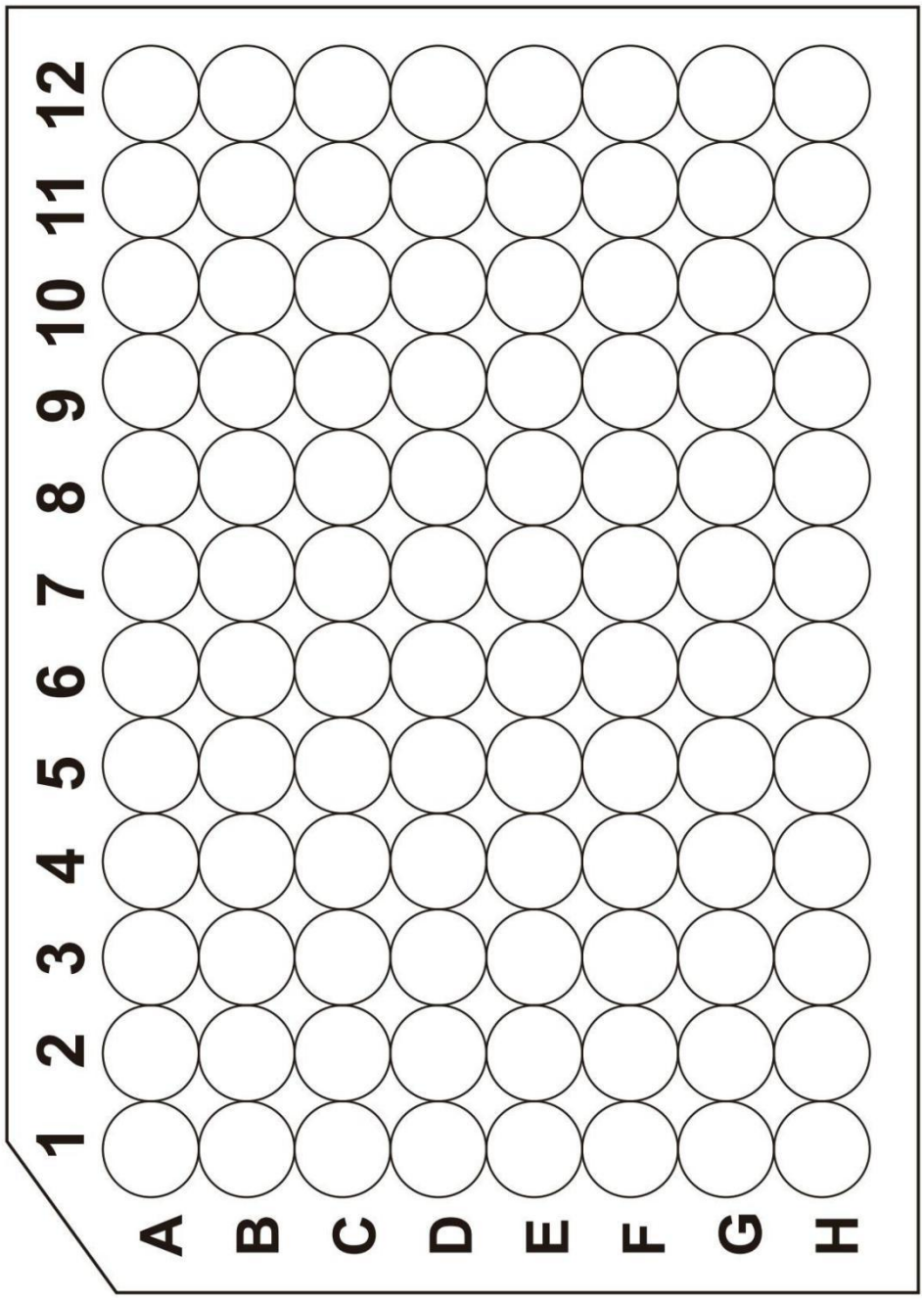
PLATE LAYOUT

Use this plate layout to record standards and samples assayed.



产品信息及操作手册

大鼠 IL-2 Valukine™ ELISA 试剂盒

目录号：VAL907

适用于定量检测天然和重组大鼠白介素 2(**IL-2**)的浓度

科研专用，不可用于临床诊断

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目录

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，属于常见的γ链（γ_c）细胞因子家族（1-4）。它以单体形式存在，半衰期极短（< 30 分钟）（1）。大鼠 IL-2 以 155 个氨基酸（amino acid, aa）前体的形式合成，其中包含 20 aa 的信号序列和 135 aa 的成熟区（5, 6）。成熟区呈α-螺旋状，在 Thr3 处含有一个利用的 O-连接糖基化位点，外加三个半胱氨酸，其中两个半胱氨酸形成链内二硫键，对其活性至关重要（7）。成熟大鼠 IL-2 与人和小鼠 IL-2 的 aa 一致性分别为 66%和 73%。尽管人 IL-2 与高度多态性小鼠 IL-2 仅具有约 60%的 aa 一致性，但已知人 IL-2 在小鼠 IL-2 反应细胞上具有活性。据报道分泌 IL-2 的细胞包括γδ T 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 受体（The receptor for IL-2, IL-2 R）由三个亚基组成，即 55 kDa CD25/IL-2 Rα链、70 kDa IL-2 Rβ链和 65 kDa γ_c（1, 3）。IL-2 首先与 CD25 结合；随后，二元复合物招募 IL-2 Rβ和γ_c形成四元信号复合物（1, 14）。除 IL-2 外，IL-15 在其四级信号复合体中也使用 IL-2 Rβ。γ_c也是 IL-4、-7、-9、-15 和-21 的信号受体（1, 3）。

体外研究表明，IL-2 在 T 细胞活化和扩增中发挥着重要作用。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 细胞可提供对自身免疫性疾病的保护。另一方面，IL-2 还能通过调节 T 细胞运输基因的表达和 Th2 细胞因子的产生来促进靶器官的自身免疫炎症。在 $CD8^+$ T 细胞亚群中，IL-2 对优化初级反应和分化为终端效应细胞至关重要。IL-2 还能促进活化的 $CD8^+$ T 细胞发育成记忆细胞（1）。

II. 概述

A. 检测原理

本实验采用双抗体夹心ELISA法。抗大鼠IL-2抗体包被于微孔板上。样品，质控品和标准品中的大鼠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大鼠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，大鼠血清样本和大鼠血浆样本；
- 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RD5-4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，在不同板间分别检测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)	130	478	1491	139	495	1550
标准差	4.2	10.5	31.9	13.8	30.8	85.4
CV%	3.2	2.2	2.1	9.9	6.2	5.5

B. 回收率

在不同类别样本中掺入检测范围内不同水平的大鼠IL-2，测定其回收率。

样本类型	平均回收率%	范围 (%)
细胞培养上清 (n=8)	110	93-120
大鼠血清* (n=14)	96	83-114
大鼠EDTA血浆* (n=4)	102	90-117
大鼠肝素血浆* (n=4)	100	92-116

*样品在分析前按照样品制备部分的指示进行稀释。

C. 灵敏度

大鼠 IL-2的最低可测剂量（MDD）通常低于15 pg/mL。

MDD是根据20个重复的零标准品孔的吸光度值的平均值加两倍标准差计算得到的相对应浓度。

D. 校正

该免疫测定法以R&D Systems生产的高纯度的大肠杆菌表达的重组大鼠IL-2校正。

E. 线性

不同的样本类型（至少含有4个样本）中含有或掺入高浓度的大鼠IL-2，然后用标准品稀释液RD5-4将其稀释并测定其线性。

样本	稀释倍数	观测值 (pg/mL)	期望值 (pg/mL)	观测值 / 期望值 x 100
细胞培养上清	Neat	1627	____	____
	1:2	851	814	105%
	1:4	413	407	101%
	1:8	210	204	103%
	1:16	101	102	99%
大鼠血清*	Spiked	1057	____	____
	1:2	557	528	105%
	1:4	286	264	108%
	1:8	132	132	100%
	1:16	65	66	98%
大鼠 EDTA 血浆*	Spiked	876	____	____
	1:2	462	438	106%
	1:4	238	219	109%
	1:8	127	110	115%
	1:16	64	55	116%
大鼠肝素血浆*	Spiked	1023	____	____
	1:2	561	512	110%
	1:4	279	256	109%
	1:8	131	128	102%
	1:16	68	64	106%

*按照样品制备部分中的指示，在检测前对样品进行加标，然后稀释。

F. 样本预值

大鼠血清样本：使用本试剂盒检测了 40 份大鼠血清中 IL-2 的水平。39 个样本的测量值低于最低标准，即 31.3 pg/mL。一个样本检测值为 238 pg/mL。

大鼠血浆样本：使用本试剂盒检测了 20 份大鼠 EDTA 血浆和 14 份大鼠肝素血浆中 IL-2 的水平。大多数检测值低于最低标准，即 31.3 pg/mL。两个 EDTA 血浆样本检测值分别为 80 pg/mL 和 92 pg/mL。一个肝素血浆样本检测值为 62 pg/mL。

细胞培养上清样本：

将大鼠脾细胞（1 x 10^7 cells/mL）培养在含 10%胎牛血清的 RPMI 培养基中，培养 3 天，然后用 5.0 µg/mL Concanavalin A 刺激。取出等分的细胞培养上清，检测大鼠 IL-2，检测值为 14 ng/mL。

G. 特异性

此 ELISA 法可检测天然及重组大鼠 IL-2。

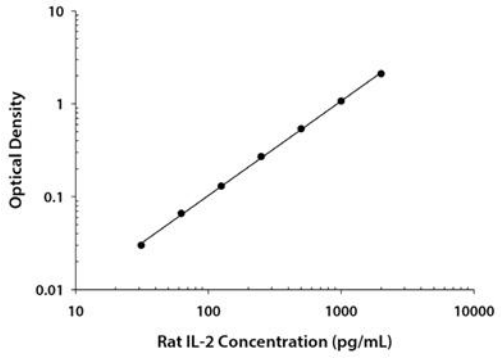
将以下因子用标准品稀释液 RD5-4 配制成 50 ng/m 的浓度来检测与大鼠 IL-2 的交叉反应。将 50 ng/mL 的干扰因子掺入中间范围的重组大鼠 IL-2 对照品中，来检测对大鼠 IL-2 的干扰。没有观察到明显的交叉反应或干扰。

Recombinant rat:		**Recombinant mouse:**
CINC-1	IL-4	IL-2
GDNF	β-NGF	**Recombinant human:**
IFN-γ	PDGF-BB	IL-2
IL-1α	TNF-α	IL-2 Rα
		IL-2 Rβ

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	0.034 0.034	0.034	—
31.3	0.065 0.063	0.064	0.030
62.5	0.105 0.096	0.100	0.066
125	0.166 0.163	0.164	0.130
250	0.308 0.300	0.304	0.270
500	0.574 0.571	0.572	0.538
1000	1.118 1.087	1.102	1.068
2000	2.161 2.117	2.139	2.105

V. 试剂盒组成及储存

A. 试剂盒组成

组成	描述	规格
Rat IL-2 Microplate	包被抗大鼠IL-2抗体的96孔聚苯乙烯板，8孔× 12条	1块板
Rat IL-2 Conjugate	酶标检测抗大鼠IL-2抗体	1瓶
Rat IL-2 Standard	大鼠IL-2标准品（冻干），参考瓶身标签进行重溶	1瓶
Rat IL-2 Control	大鼠IL-2质控品（冻干），质控品的测定值应在标签上规定的范围内	1瓶
Assay Diluent RD1-21	检测液	1瓶
Calibrator Diluent RD5-4	标准品稀释液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	2瓶
Stop Solution	终止液	1瓶
Plate Sealers	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RD1-21</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>标准品稀释液RD5-4</td><td rowspan="2">每次实验使用新的标准品与质控品，使用后丢弃。</td></tr>
<tr><td>质控品</td></tr>
<tr><td>标准品</td><td rowspan="2">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>
<tr><td></td><td>包被的微孔板条</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
- 用于稀释标准品和样品管子

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. 样本收集及储存

以下列出的样品收集和储存条件仅作为一般指南。样本稳定性尚未评估。

细胞培养上清：颗粒物应通过离心去除；立即检测样本或分装，≤-20 °C储存备用，避免反复冻融。样本可能需要用标准品稀释液RD5-4稀释。

血清样本：血液样本在室温下凝集2小时，然后在1000 × g下离心20分钟。吸取血清样本之后即刻用于检测，或者分装，≤ -20°C储存备用。避免反复冻融。样本可能需要用标准品稀释液RD5-4稀释。

血浆样本：使用EDTA与肝素作为抗凝剂收集血浆。然后1000 × g离心20分钟。需在30分钟内收集血浆样本之后即刻用于检测，或者分装，≤-20°C储存备用。避免反复冻融。样本可能需要用稀释液RD5-4稀释。

注意： *本试剂盒对枸橼酸钠血浆尚未被验证。*

本试剂盒不适合用于溶血或血脂过高样本。

B. 样本准备工作

大鼠血清和血浆样本建议用标准品稀释液RD5-4 2倍稀释后进行检测，即75 µL样品+75 µL标准品稀释液RD5-4。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。

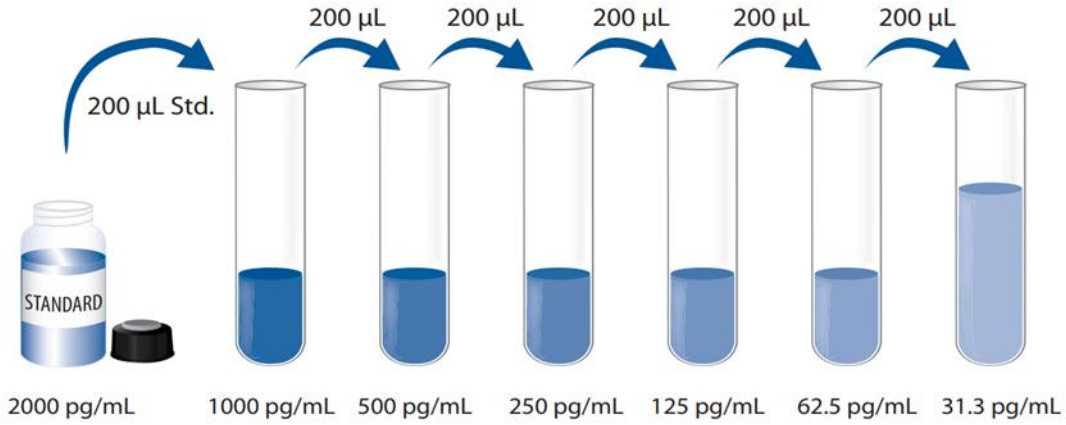
大鼠IL-2质控品：使用1.0 mL去离子水或蒸馏水重溶质控品。混合均匀，测定时不稀释质控品。

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×）。

大鼠IL-2标准品：重溶体积请参考瓶身标签*，用标准品稀释液RD5-4重溶大鼠IL-2标准品，请勿使用其他稀释液。得到浓度为2000 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。

向每一稀释管中加入200 µL标准品稀释液RD5-4。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。未稀释的大鼠 IL-2 标准品可用作标准曲线最高点（2000 pg/mL），标准品稀释液RD5-4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，质控品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，标准品，质控品和样本；
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 50μL 检测液 RD1-21。
4. 分别将不同浓度标准品，质控品和实验样本加入相应孔中，每孔 50 μL。轻轻拍打微孔板 1 分钟，后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 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 大鼠 IL-2 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，质控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 log/log 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 log/log 图上的点绘制最佳拟合曲线。数据可以通过绘制大鼠 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。

    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