PRODUCT INFORMATION & MANUAL

Human IL-2 Valukine™ ELISA

VAL110

For the quantitative determination of natural and recombinant human 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

Version202209.4

TABLE OF CONTENTS

I. BACKGROUND

Human 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). Human IL-2 is synthesized as a 153 amino acid (aa) precursor that contains a 20 aa signal sequence plus a 133 aa mature region (5, 6). The mature region 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 human IL-2 shares 73%, 66%, 78% and 97% aa sequence identity with canine, rat, feline and rhesus monkey IL-2, respectively. Although human IL-2 shares only approximately 60% aa identify 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-2R) is composed of three subunits, the 55 kDa CD25/IL-2Rα chain, the 70 kDa CD122/IL-2Rβ chain, and the 65 kDa CD132/ γc chain (1, 3). IL-2 first binds to CD25, the binary complex then recruits CD122 and CD132 to form the quaternary signaling complex (1, 14). In addition to IL-2, CD122/IL-2Rβ is used by IL-15 in its quaternary signaling complex. CD132/ 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. A monoclonal antibody specific for IL-2 has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any IL-2 present is bound by the immobilized antibody. After washing away any unbound substances, an enzyme-linked polyclonal antibody specific for 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 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 ROCEDURES.**
- 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.

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.

CELL CULTURE SUPERNATE / SERUM

	Intra-assay Precision			**Inter-assay Precision**		
Sample	1	2	3	1	2	3
Mean (pg/mL)	162	435	809	170	470	897
Standard Deviation	6.4	17.3	28.5	11.7	28.4	49.0
CV%	3.9	4.0	3.5	6.9	6.0	5.5

B. RECOVERY

The recovery of human IL-2 spiked to different levels throughout the range of the assay in cell culture media was evaluated. The recovery ranged from 98-110% with an average of 106%.

The recovery of human IL-2 spiked to different levels throughout the range of the assay in human serum was evaluated. The recovery ranged from 77.1-95.0% with an average of 81.7%.

C. SENSITIVITY

The minimum detectable dose (MDD) of IL-2 is typically less than 15.6 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 human IL-2 produced at R&D Systems. The NIBSC/WHO 2nd International Standard for IL-2 (86/500), which was intended as a potency standard, was evaluated in this kit. The NIBSC/WHO standard is *E. coli* -derived recombinant human IL-2.

The dose response curve of the International Standard (86/500) parallels the Valukine standard curve. To convert sample values obtained with the Valukine Human IL-2 kit to approximate NIBSC 86/500 units, use the equation below.

NIBSC (86/500) approximate value (IU/mL) = 0.022 × Valukine Human IL-2 value (pg/mL)

E. LINEARITY

To assess the linearity of the assay, samples were spiked with high concentrations of IL-2 and diluted with Calibrator Diluent (*for cell culture supernate samples*) or Calibrator Diluent-S (*for serum samples*) to produce samples with values within the dynamic range of the assay.

Dilution	Average % of Expected	Range (%)
1:2	104	101 – 107
1:4	106	101 – 110
1:8	110	101 – 115
1:16	112	99 – 122

F. SAMPLE VALUES

Cell Culture Supernates - Human peripheral blood mononuclear cells (1×10^6 cells/mL) were cultured in RPMI supplemented with 10% fetal calf serum, 50 µM β-mercaptoethanol, 2 mM L-glutamine, 100 U/mL penicillin, and 100 µg/mL streptomycin sulfate and stimulated for 24 hours with 10 µg/mL PHA. An aliquot of the cell culture supernate was removed, assayed for levels of natural IL-2, and measured 2172 pg/mL.

Serum - Four serum samples were evaluated for the presence of IL-2 in this assay. All samples measured below the lowest standard, 31.3 pg/mL.

G. SPECIFICITY

This assay recognizes both natural and recombinant human IL-2. The following factors were prepared at 100 ng/mL and assayed for cross-reactivity. Preparations of the following factors at 100 ng/mL in a mid-range rhIL-2 control were assayed for interference. No significant cross-reactivity or interference was observed.

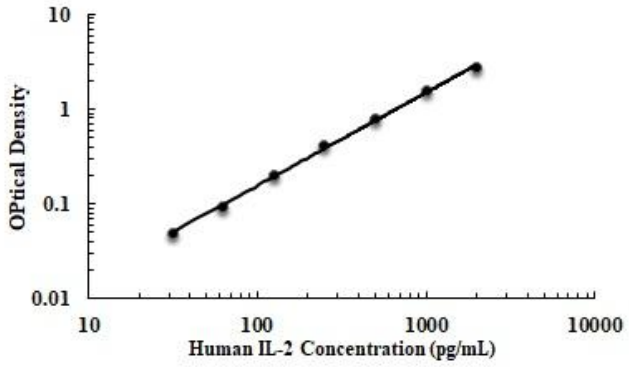
Recombinant human	Recombinant mouse
IL-2 sRα	IL-2
IL-2 Rβ	IL-4
IL-2 Rγ	
IL-4	

IV. EXPERIMENT

A. EXAMPLE STANDARD

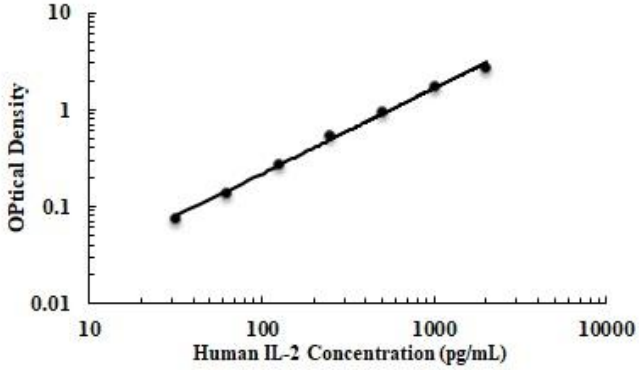
The standard curve is provided for demonstration only. A standard curve should be generated for each set of samples assayed.

CELL CULTURE SUPERNATE ASSAY



pg/ml	OD	Average	Corrected
0	0.016 0.017	0.017	-
31.3	0.066 0.066	0.066	0.049
62.5	0.110 0.111	0.111	0.094
125	0.212 0.213	0.213	0.196
250	0.417 0.431	0.424	0.407
500	0.789 0.819	0.804	0.787
1000	1.557 1.562	1.560	1.543
2000	2.728 2.741	2.735	2.718

SERUM ASSAY



pg/ml	OD	Average	Corrected
0	0.027 0.029	0.028	-
31.3	0.097 0.108	0.103	0.075
62.5	0.158 0.169	0.164	0.136
125	0.290 0.315	0.303	0.275
250	0.578 0.550	0.564	0.536
500	1.003 0.972	0.988	0.960
1000	1.723 1.846	1.785	1.757
2000	2.688 2.750	2.719	2.691

V. KIT COMPONENTS AND STORAGE

A. MATERIALS PROVIDED

Parts	Description	Size
IL-2 Microplate	96 well polystyrene microplate (12 strips of 8 wells) coated with a mouse monoclonal antibody against human IL-2	1 plate
IL-2 Conjugate	solution of polyclonal antibody against IL-2 conjugated to horseradish peroxidase	1 vial
IL-2 Standard	recombinant human IL-2 in a buffered protein base; lyophilized	1 vial
Calibrator Diluent (5×)	a 5× concentrated buffered protein base used to dilute standard and cell culture supernate samples	1 vial
Calibrator Diluent-S (1×)	a buffered protein base used to dilute standard and serum samples	1 vial
Wash Buffer Concentrate(25×)	a 25× concentrated solution of buffered surfactant	1 vial
TMB Substrate	TMB ELISA Substrate Solution	2 vials
Stop Solution	2 N sulfuric acid	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="7">Opened/ Reconstituted Reagents</td><td>Diluted Wash Solution</td><td rowspan="5">May be stored for up to 1 month at 2-8°C.*</td></tr>
<tr><td>Stop Solution</td></tr>
<tr><td>Calibrator Dilution (1×)</td></tr>
<tr><td>Calibrator Dilution-S (1×)</td></tr>
<tr><td>Conjugate</td></tr>
<tr><td>TMB Substrate</td><td rowspan="2">Aliquot and store for up to 1 month at <-20°C in a manual defrost freezer. * Avoid repeated freeze-thaw cycles.</td></tr>
<tr><td>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. 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 ℃. Avoid repeated freeze-thaw cycles. Samples may require dilution with Calibrator Diluent (1×).

Serum - Use a serum separator tube (SST) and allow samples to clot for 30 minutes at room temperature before centrifugation for 15 minutes at 1000 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 2-fold dilution. A suggested 2-fold dilution is 100 µL of sample + 100 µL of Calibrator Diluent-S (1×).

C. REAGENT PREPARATION

Note: Bring all reagents to room temperature before use.

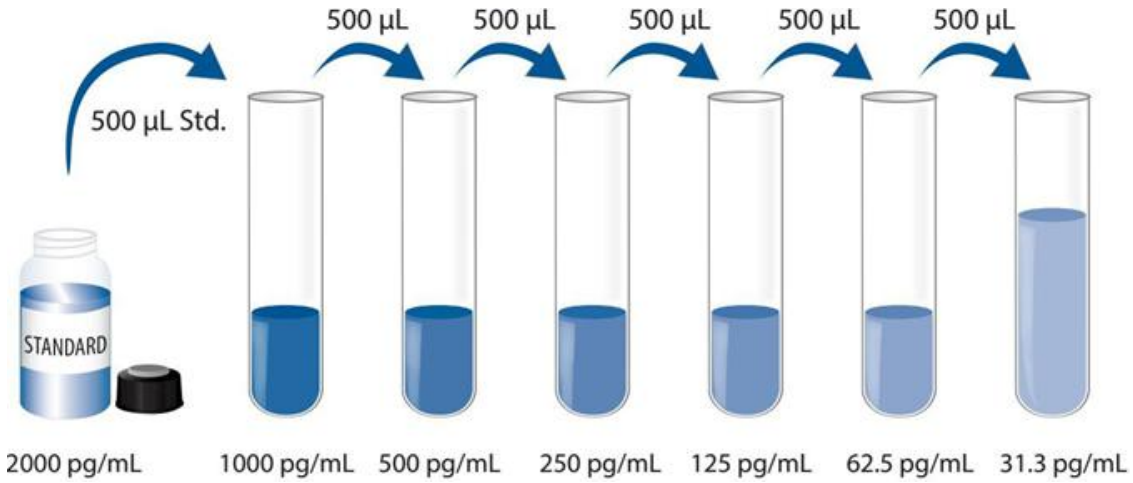
Wash Solution - If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. Dilute 20 mL of Wash Buffer Concentrate (25×) into deionized or distilled water to prepare 500 mL of Wash Buffer.

Calibrator Diluent (1×) - Add 20 mL of Calibrator Diluent (5×) into 80 mL of deionized or distilled water to prepare 100 mL of Calibrator Diluent (1×).

IL-2 Standard- Refer to the vial label for the reconstitution volume* using Calibrator Diluent (1×) (*for cell culture supernate samples*) or Calibrator Diluent-S (1×) (*for serum samples*). 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 500µL of Calibrator Diluent (1×) (*for cell culture supernate samples*) or Calibrator Diluent-S (1×) (*for serum samples)* into each tube. Use the stock solution to produce a dilution series (below). Mix each tube thoroughly before the next transfer. The undiluted standard serves as the high standard (2000 pg/mL). The Calibrator Diluent (1×) (*for cell culture supernate samples*) or Calibrator Diluent-S (1×) (*for serum samples*) 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 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.

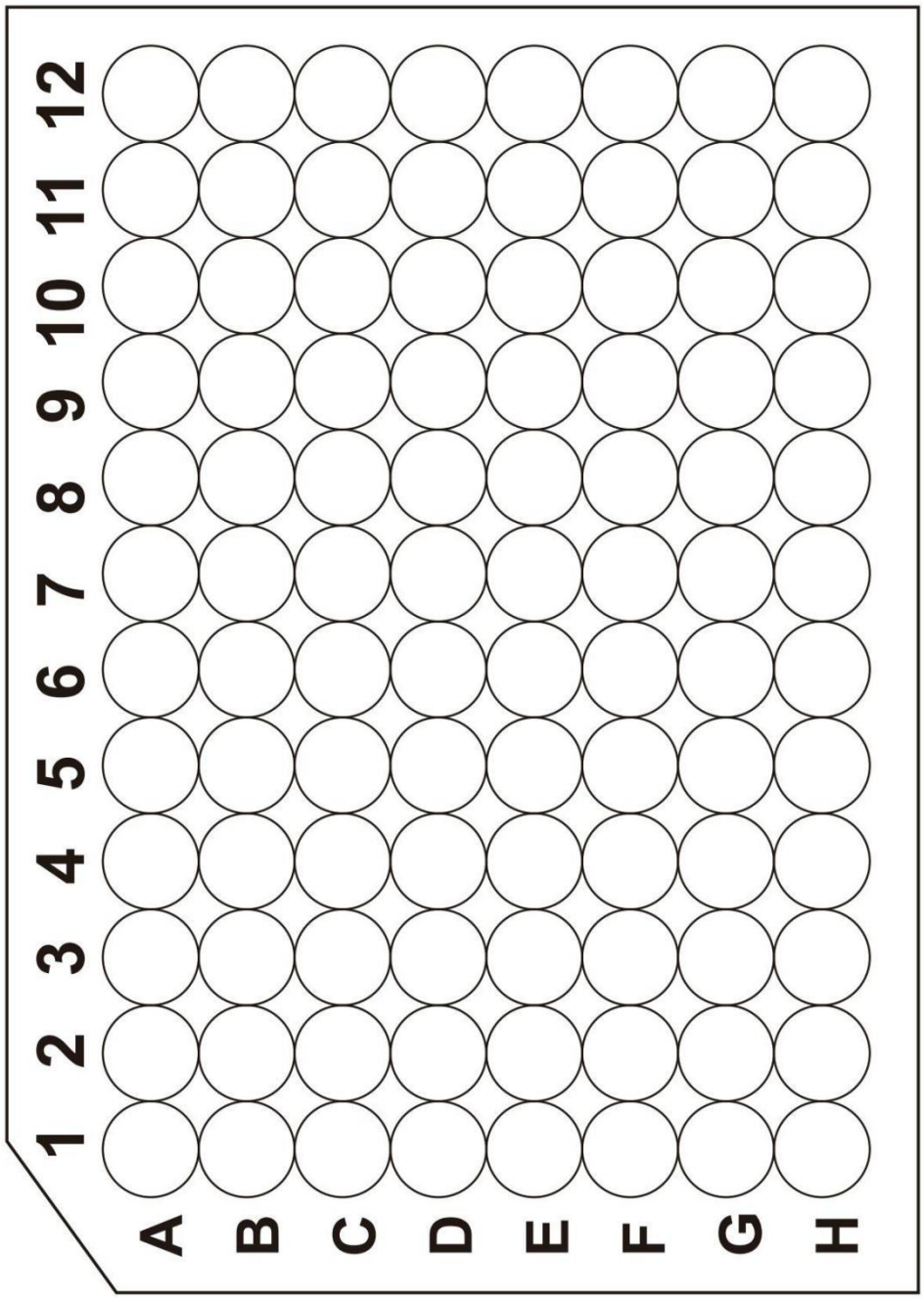
1. Prepare all reagents and working standards as directed in the previous sections.
2. Remove excess microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, and reseal.
3. Add 100 µL of Standard, sample, or control per well. Cover with the adhesive strip provided. Incubate for 2 hours at room temperature. A plate layout is provided for a record of standards and samples assayed.
4. 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.
5. Add 200 µL of IL-2 Conjugate to each well. Cover with a new adhesive strip. Incubate for 2 hours at room temperature.
6. Repeat the aspiration/wash as in step 4.
7. Add 200 µL of TMB Substrate to each well. Incubate for 30 minutes at room temperature. **Protect from light**.
8. Add 50 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.
9. 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.
10. **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-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. Taniguchi, T. et al. (1983) Nature 302:305.
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

人 IL-2 Valukine™ ELISA 试剂盒

目录号：VAL110

适用于定量检测天然和重组人白介素 2（IL-2）的含量

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

目录

I. 背景

人白细胞介素2（IL-2），又称为T细胞生长因子（TCGF），是一个15-18 kDa大小的具有不同糖基化的α-螺旋多肽，属于常见的γ链（γc）细胞因子家族成员（1-4）。它以单体存在，半衰期很短（<30分钟）（1）。人IL-2的前体有153个氨基酸，其中包含一个20个氨基酸的信号序列，和一个133个氨基酸的成熟多肽（5, 6）。IL-2成熟蛋白包含一个在3位苏氨酸可O型糖基化的位点和三个半胱氨酸，其中的两个半胱氨酸所形成的链内二硫键是IL-2活性所必不可少的（7）。成熟的人IL-2氨基酸序列与犬、大鼠、猫和猕猴的IL-2同源性分别为73%、66%、78%和97%。虽然人IL-2与高度多态的小鼠IL-2仅有60%的氨基酸同源性，但人IL-2对小鼠细胞也具有生物活性。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）、造血干细胞（13）。

IL-2受体（IL-2R）由三个亚基构成，即一个55 kDa的CD25/IL-2Rα链、一个70 kDa的CD122/IL-2Rβ链，以及一个65 kDa的CD132/γc链（1, 3）。IL-2先与CD25结合，形成的二亚基复合物再招募CD122和CD132，形成具有四个亚基的信号复合物（1, 14）。除了能与IL-2形成复合物，CD122/IL-2Rβ也可同IL-15形成四亚基信号复合物。CD132/c亦可成为IL-4、IL-7、IL-9、IL-15和IL-21信号的受体（1, 3）。

体外研究表明，IL-2在T细胞活化和扩增中起重要作用。在体内，IL-2是调节性T细胞（Treg）发育、维持和功能的关键，而调节性T细胞可为自身免疫性疾病提供保护。此外，IL-2也可通过调节T细胞贩运基因的表达和Th2型细胞因子的产生，从而促进其靶器官自身免疫性炎症。在$CD8^+$T细胞亚群中，IL-2是获得最佳一级反应和分化到终端效应细胞的必要关键。IL-2也促进了$CD8^+$T细胞的发育和激活成为记忆细胞（1）。

II. 概述

A. 检测原理

本实验采用双抗体夹心ELISA法。抗人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人IL-2多抗，与结合在微孔板上的IL-2结合而形成免疫复合物，游离的成分被洗去；加入TMB底物溶液，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。

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。

细胞培养上清/血清

	板内精确度			板间精确度		
样本	1	2	3	1	2	3
平均值 (pg/mL)	162	435	809	170	470	897
标准差	6.4	17.3	28.5	11.7	28.4	49.0
CV%	3.9	4.0	3.5	6.9	6.0	5.5

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人IL-2，测定其回收率。回收率范围在98-110%，平均回收率在106%。

在人血清样本中掺入检测范围内不同水平的人IL-2，测定其回收率。回收率范围在77.1-95.0%，平均回收率在81.7%。

C. 灵敏度

人IL-2的最低可测剂量（MDD）一般小于15.6 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. 标定

此ELISA试剂盒是针对R&D Systems生产的大肠杆菌表达的高纯度重组人IL-2进行校准的。NIBSC/WHO IL-2第2国际标准品(86/500)作为效价标准，在本试剂盒中进行了评估。NIBSC/WHO标准品是大肠杆菌来源的重组人IL-2。

国际标准品(86/500)的剂量反应曲线与Valukine标准曲线平行。若要将使用Valukine Human IL-2 kit获得的样本值转换为NIBSC 86/500的近似单位，请使用以下公式：

NIBSC (86/500) approximate value (IU/mL) = 0.022 × Valukine Human IL-2 value (pg/mL)

E. 线性

不同的细胞上清/人血清中掺入高浓度的人IL-2，然后用稀释剂（1×）（*用于细胞上清样本*）或稀释剂-S（1×）（*用于人血清样本*）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	104	101 – 107
1:4	106	101 – 110
1:8	110	101 – 115
1:16	112	99 – 122

F. 样本预值

细胞上清样本-人的外周血单核细胞（1×10^6细胞/mL）培养于含有10%胎牛血清的RPMI1640 培养基中，细胞培养基还含有2mML-谷氨酰胺、50μMβ-巯基乙醇、100U/mL青霉素、100μg/mL链霉素，另加10μg/mL PHA刺激细胞，培养24小时。取细胞上清液测定IL-2含量，结果为2172pg/mL。

血清样本-使用本试剂盒检测了4份人血清样本中IL-2的水平。4份样本的检测值均低于人IL-2最低标准品，31.3pg/mL。

G. 特异性

此ELISA法可检测天然及重组人IL-2蛋白。将以下因子用稀释剂（1×）配制成100ng/mL的浓度来检测与人IL-2的交叉反应。将100ng/mL的干扰因子掺入中间范围的重组人IL-2对照品中，来检测对人IL-2的干扰。没有观察到明显的交叉反应或干扰。

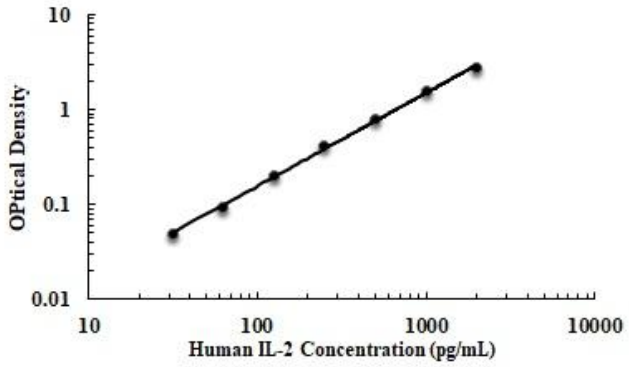
重组人蛋白	重组小鼠蛋白
IL-2 sRα	IL-2
IL-2 Rβ	IL-4
IL-2 Rγ	
IL-4	

IV. 实验标准

A. 标准曲线实例

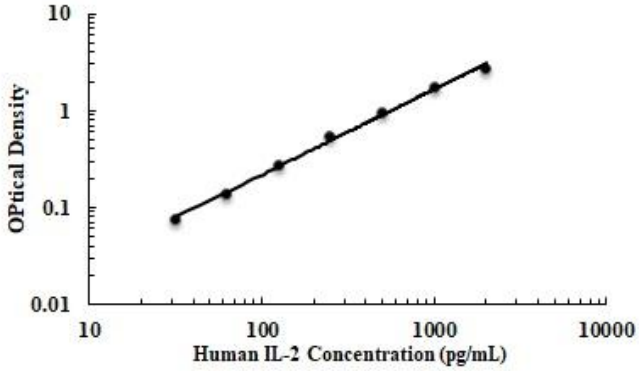
该标准曲线数据仅供参考，每次实验应绘制其对应的标准曲线。

CELL CULTURE SUPERNATE ASSAY



pg/ml	OD	Average	Corrected
0	0.016 0.017	0.017	-
31.3	0.066 0.066	0.066	0.049
62.5	0.110 0.111	0.111	0.094
125	0.212 0.213	0.213	0.196
250	0.417 0.431	0.424	0.407
500	0.789 0.819	0.804	0.787
1000	1.557 1.562	1.560	1.543
2000	2.728 2.741	2.735	2.718

SERUM ASSAY



pg/ml	OD	Average	Corrected
0	0.027 0.029	0.028	-
31.3	0.097 0.108	0.103	0.075
62.5	0.158 0.169	0.164	0.136
125	0.290 0.315	0.303	0.275
250	0.578 0.550	0.564	0.536
500	1.003 0.972	0.988	0.960
1000	1.723 1.846	1.785	1.757
2000	2.688 2.750	2.719	2.691

V. 试剂盒组成及储存

B. 试剂盒组成

组成	描述	规格
IL-2 Microplate	包被抗体的96孔聚苯乙烯板，8孔× 12条	1块板
IL-2 Conjugate	酶标检测IL-2抗体	1瓶
IL-2 Standard	标准品（冻干）	1瓶
Calibrator Diluent（5×）	浓缩稀释剂（5×），用于稀释标准品和细胞上清样本	1瓶
Calibrator Diluent-S（1×）	稀释剂-S（1×），用于稀释标准品和血清样本	1瓶
Wash Buffer Concentrate（25×）	浓缩洗涤缓冲液（25×）	1瓶
TMB Substrate	TMB底物溶液	2瓶
Stop Solution	终止液	1瓶

C. 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>稀释剂-S（1×）</td></tr>
<tr><td>酶标检测抗体</td></tr>
<tr><td>TMB底物溶液</td></tr>
<tr><td>标准品</td><td>分装，-20℃以下冰箱储存最多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

D. 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

E. 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1000xg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-S（1×）2倍稀释后进行检测，即100µL血清+100µL稀释剂-S（1×）。

C. 检测前准备工作

使用前请将所有试剂放置于室温

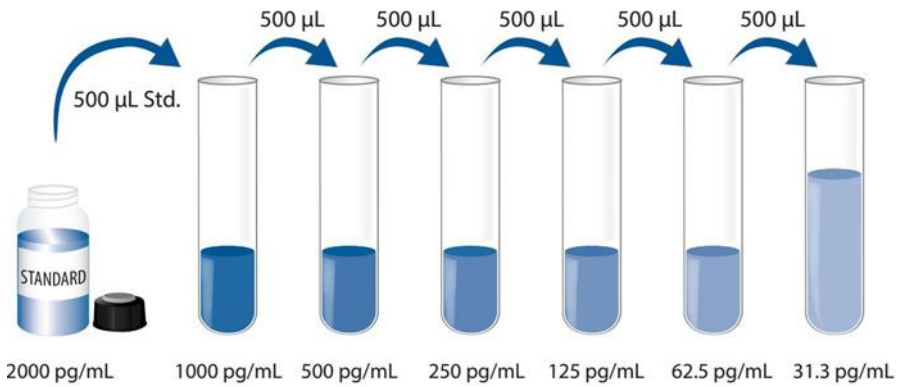
洗涤液：从冰箱中取出的浓缩洗涤液可能有结晶，属于正常现象；放置室温，轻摇混匀，待结晶完全溶解后再配制洗涤液。可将20 mL浓缩洗涤液用蒸馏水或去离子水稀释配制成500mL工作浓度的洗涤液。

稀释剂（1×）：可将20mL浓缩稀释剂（5×）用80mL蒸馏水或去离子水稀释配制成100mL工作浓度的稀释剂（1×）。

标准品：重溶体积请参考瓶身标签，稀释剂（1×）（*用于细胞上清样本*）或稀释剂-S（1×）（*用于人血清样本*），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。

每个稀释管中加入500 µL稀释剂（1×）（*用于细胞上清样本*）或稀释剂-S（1×）（*用于人血清样本*）。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（2000 pg/mL），稀释剂（1×）（*用于细胞上清样本*）或稀释剂-S（1×）（*用于人血清样本*）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，正确使用封板胶纸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. 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100μL。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；
4. 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；
5. 在每个微孔内加入200μL酶标检测抗体。用封板胶纸封住反应孔，室温孵育2小时；
6. 重复第4步洗板操作；
7. 在每个微孔内加入200μL TMB底物溶液，室温孵育30分钟。注意避光；
8. 在每个微孔内加入50μL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；
9. 加入终止液后10分钟内，使用酶标仪测量450nm的吸光度值，设定540nm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；
10. 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

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. Taniguchi, T. et al. (1983) Nature 302:305.
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