



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.

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

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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 (γ c) 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 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 $\gamma\delta$ 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-2R α chain, the 70 kDa CD122/IL-2R β chain, and the 65 kDa CD132/ γ c 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-2R β is used by IL-15 in its quaternary signaling complex. CD132/ γ c 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 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.

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

Sample	Intra-assay Precision			Inter-assay Precision		
	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 μ M β -mercaptoethanol, 2 mM L-glutamine, 100 U/mL penicillin, and 100 μ g/mL streptomycin sulfate and stimulated for 24 hours with 10 μ g/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.

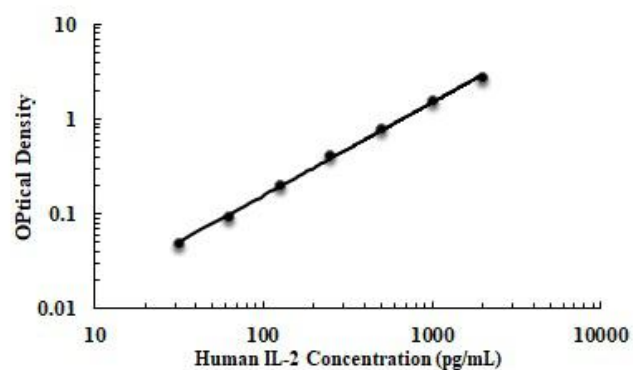
Recombinant human	Recombinant mouse
IL-2 sR α	IL-2
IL-2 R β	IL-4
IL-2 R γ	
IL-4	

IV. EXPERIMENT

A. EXAMPLE STANDARD

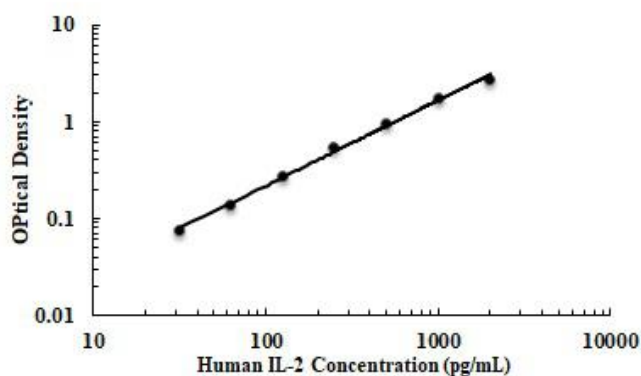
The standard curve is provided for demonstration only. A standard curve should be generated for each set of samples assayed.

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
Color Reagent A	stabilized hydrogen peroxide	1 vial
Color Reagent B	stabilized chromogen (tetramethylbenzidine)	1 vial
Stop Solution	2 N sulfuric acid	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	
	Calibrator Dilution (1×)	
	Calibrator Dilution-S (1×)	
	Conjugate	
	Unmixed Substrate A	
	Unmixed Substrate B	
	Standard	Aliquot and store for up to 1 month at <-20°C in a manual defrost freezer. * Avoid repeated freeze-thaw cycles.
Microplate Wells	Return unused wells to the 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.*	

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

C. OTHER SUPPLIES REQUIRED

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

D. PRECAUTION

The Stop Solution provided with this kit is an acid solution. Wear eye, hand, face, and clothing protection when using this material.

VI. PREPARATION

A. SAMPLE COLLECTION AND STORAGE

Cell Culture Supernates - Remove particulates by centrifugation and assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles. 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 μ L of sample + 100 μ L 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.

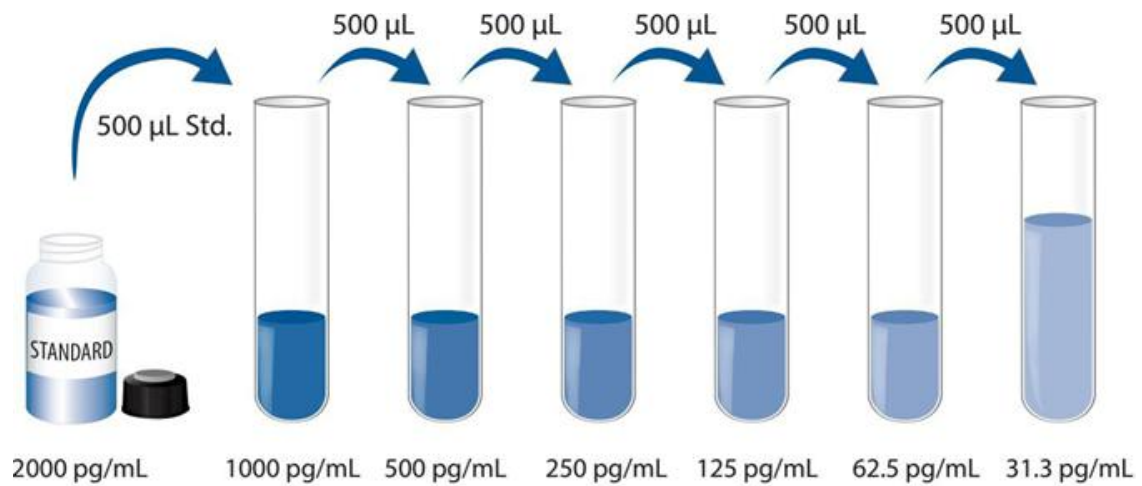
Substrate Solution - Substrates A and B should be mixed together in equal volumes within 15 minutes of use. Protect from light. 200 μ L of the resultant mixture is required per well.

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 500 μ L 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.
- Substrate Solution should remain colorless until added to the plate. Keep Substrate Solution protected from light. Substrate Solution should change from colorless to gradations of blue.
- Stop Solution should be added to the plate in the same order as the Substrate Solution. The color developed in the wells will turn from blue to yellow upon addition of the Stop Solution. Wells that are green in color indicate that the Stop Solution has not mixed thoroughly with the Substrate Solution.

VII. ASSAY PROCEDURE

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

1. Prepare all reagents and working standards as directed in the previous sections.
2. Remove excess microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, and reseal.
3. Add 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 Substrate Solution 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 30 minutes, using a microplate reader set to 450 nm. If wavelength correction is available, set to 540 nm or 570 nm. If wavelength correction is not available, subtract readings at 540 nm or 570 nm from the readings at 450 nm. This subtraction will correct for optical imperfections in the plate. Readings made directly at 450 nm without correction may be higher and less accurate.
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

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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

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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

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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 (γ c) 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 $\gamma\delta$ 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-2R α 链、一个 70 kDa 的 CD122/IL-2R β 链，以及一个 65 kDa 的 CD132/ γ c 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-2R β 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；加入辣根过氧化物酶标记的抗人 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；加入底物溶液（显色剂），溶液颜色逐渐变成蓝色，加入终止液溶液变黄并且停止变化。用酶标仪测定吸光度。

B. 检测局限

- ◆ 仅供科研使用，不可用于体外诊断；
- ◆ 该试剂盒适用于细胞培养上清样本和血清样本；
- ◆ 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；若细胞培养上清液样本需分步稀释，除最后一步用稀释剂稀释外，其它中间稀释可采用细胞培养基；
- ◆ 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

样本	板内精确度			板间精确度		
	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 2 mM L-谷氨酰胺、50 μ M β -巯基乙醇、100 U/mL 青霉素、100 μ g/mL 链霉素, 另加 10 μ g/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 2172 pg/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最低检测限, 31.3 pg/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 \times) 配置成 100 ng/mL的浓度来检测与人 IL-2 的交叉反应。将 100 ng/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。

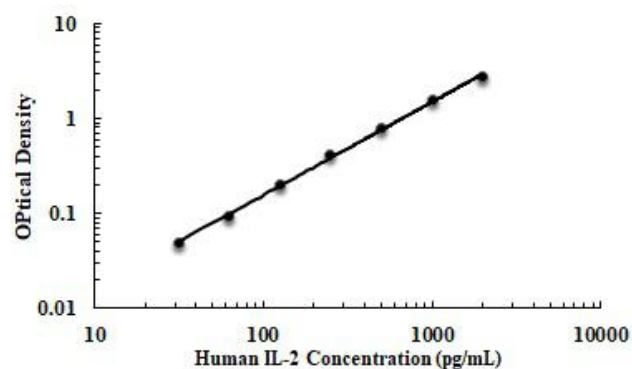
重组人蛋白	重组小鼠蛋白
IL-2 sR α	IL-2
IL-2 R β	IL-4
IL-2 R γ	
IL-4	

IV. 实验标准

A. 标准曲线实例

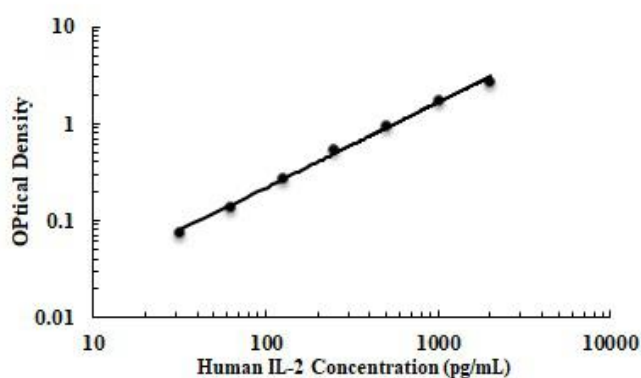
该标准曲线数据仅供参考，每次实验应绘制其对应的标准曲线。

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
Color Reagent A	显色液A	1 瓶
Color Reagent B	显色液B	1 瓶
Stop Solution	终止液	1 瓶
Plate Covers	封板胶纸	3 张

C. 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	
	稀释剂（1×）	
	稀释剂-S（1×）	
	酶标检测抗体	
	显色剂A	
	显色剂B	
	标准品	分装，-20℃ 以下冰箱储存最多 30 天*； 避免反复冻融。
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

D. 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

E. 注意事项

试剂盒中的终止液是酸性溶液，使用时请做好眼镜、手、面部及衣服的保护。

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°C 冰箱内，避免反复冻融。样本可能需要用稀释剂（ $1\times$ ）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 $1000\times g$ 离心15分钟。吸取血清样本之后即刻用于检测，或者分装， -20°C 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（ $1\times$ ）2倍稀释后进行检测，即 $100\ \mu\text{L}$ 血清 + $100\ \mu\text{L}$ 稀释剂-S（ $1\times$ ）。

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 $20\ \text{mL}$ 浓缩洗涤液用蒸馏水或去离子水稀释配置成 $500\ \text{mL}$ 工作浓度的洗涤液。未用完的放回 4°C 。

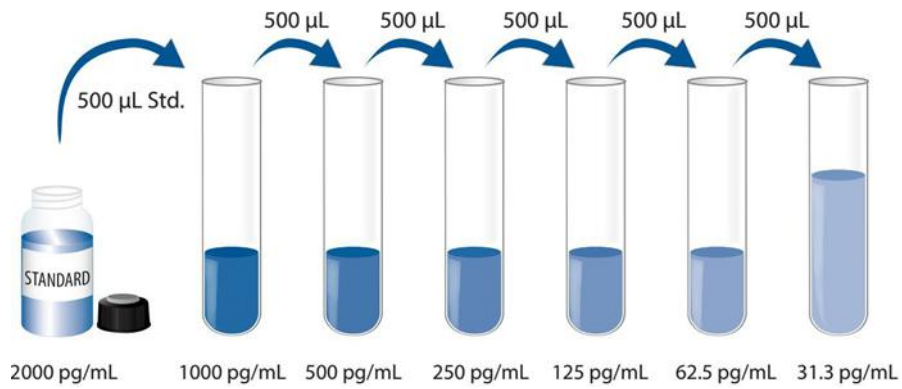
显色剂：按当次试验所需要用量将显色剂 A 和显色剂 B 等体积混合，避光；在使用前 15 分钟内准备，仅供当日使用；每孔需 $200\ \mu\text{L}$ 。

稀释剂（ $1\times$ ）：可将 $20\ \text{mL}$ 浓缩稀释剂（ $5\times$ ）用 $80\ \text{mL}$ 蒸馏水或去离子水稀释配置成 $100\ \text{mL}$ 工作浓度的稀释剂（ $1\times$ ）。

标准品：重溶体积请参考瓶身标签，稀释剂（ $1\times$ ）（用于细胞上清样本）或稀释剂-S（ $1\times$ ）（用于人血清样本），得到浓度为 $2000\ \text{pg/mL}$ 标准品母液。轻轻震荡至少 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 $500\ \mu\text{L}$ 稀释剂（ $1\times$ ）（用于细胞上清样本）或稀释剂-S（ $1\times$ ）（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（ $2000\ \text{pg/mL}$ ），稀释剂（ $1\times$ ）（用于细胞上清样本）或稀释剂-S（ $1\times$ ）（用于人血清样本）可用作标准曲线零点（ $0\ \text{pg/mL}$ ）。



D. 技术小提示

- ◆ 当混合或重溶蛋白液时，尽量避免起沫；
- ◆ 为了避免交叉污染，配置不同浓度标准品、上样、加不同试剂都需要更换枪头。另外不同试剂请分别使用不同的移液槽；
- ◆ 建议 15 分钟内完成一块板的上样；
- ◆ 每次孵育时，正确使用封板胶纸可保证结果的准确性；
- ◆ 混合后的显色底物在上板前应无色，请避光保存；加入微孔板后，将由无色变成不同深度的蓝色；
- ◆ 终止液上板顺序应同显色底物上板顺序一致；加入终止液后，孔内颜色由蓝变黄；若孔内有绿色，则表明孔内液体未混匀请充分混合

E. 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 100 μL 。用封板胶纸封住反应孔，室温孵育 2 小时。说明书提供了一张 96 孔模板图，可用于记录标准品和试验样本的板内位置；
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 400 μL ，然后将板内洗涤液吸去。重复操作 4 次。每次洗板尽量吸去残留液体会有助于得到好的实验结果。最后一次洗板结束，请将板内所有液体吸干或将板倒置，在吸水纸拍干所有残留液体；
5. 在每个微孔内加入 200 μL 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 200 μL 显色底物，室温孵育 30 分钟。注意避光；
8. 在每个微孔内加入 50 μL 终止液，孔内溶液颜色会从蓝色变为黄色。如果溶液颜色变为绿色或者颜色变化不一致，请轻拍微孔板，使溶液混合均匀；
9. 加入终止液后 30 分钟内，使用酶标仪测量 450 nm 的吸光度值，设定 540 nm 或 570 nm 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 ($\text{OD}_{450}-\text{OD}_{540}/\text{OD}_{570}$)、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。

VII. 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

