

Valukine™ ELISA Kit

Human IL-2 Immunoassay

Catalog Number: VAL110

For the quantitative determination of human interleukin 2 (IL-2) concentrations in cell culture supernates.

This kit contains sufficient materials to run an ELISA on one 96 well plate. This package insert must be read in its entirety before using this product.

**FOR RESEARCH USE ONLY.
NOT FOR USE IN DIAGNOSTIC PROCEDURES.**

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MANUFACTURED BY:

R&D Systems, Inc.
614 McKinley Place NE
Minneapolis, MN 55413
United States of America

DISTRIBUTED BY:

R&D Systems China Co. Ltd.
24A1 Hua Min Empire Plaza
726 West Yan An Road
Shanghai PRC 200050

TELEPHONE: +86 (21) 52380373
FAX: +86 (21) 52371001
E-MAIL: info@RnDSystemsChina.com.cn

INTRODUCTION

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 a 20 aa signal sequence plus a 133 aa mature region (5, 6). The mature protein 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 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 $\gamma\delta$ 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).

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.

LIMITATIONS OF THE PROCEDURE

w FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.

- w The kit should not be used beyond the expiration date on the kit label.
- w Do not mix or substitute reagents with those from other lots or sources.
- w If samples generate values higher than the highest standard, dilute the samples with Diluent and repeat the assay.
- w Any variation in operator, pipetting technique, washing technique, incubation time or temperature, and kit age can cause variation in binding.

MATERIALS PROVIDED

IL-2 Microplate - 96 well polystyrene microplate (12 strips of 8 wells) coated with a mouse monoclonal antibody against human IL-2.

IL-2 Conjugate - 21 mL of polyclonal antibody against IL-2 conjugated to horseradish peroxidase with preservatives.

IL-2 Standard - 10 ng of recombinant human IL-2 in a buffered protein base with preservatives; lyophilized.

Diluent Concentrate 5X - 21 mL of a 5X concentrated buffered protein base with preservatives.

Wash Solution Concentrate 25X - 21 mL of a 25X concentrated solution of buffered surfactant with preservatives.

Substrate A - 12.5 mL of stabilized hydrogen peroxide.

Substrate B - 12.5 mL of stabilized chromogen (tetramethylbenzidine).

Stop Solution 1 - 6 mL of 2 N sulfuric acid.

Plate Covers - 3 adhesive strips.

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 1	
	Diluent 1X	
	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.

OTHER SUPPLIES REQUIRED

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

PRECAUTION

The Stop solution 1 provided with this kit is an acid solution. Wear eye, hand, face, and clothing protection when using this material.

SAMPLE COLLECTION AND STORAGE

Cell Culture Supernates - Remove particulates by centrifugation and assay immediately or aliquot and store samples at $\leq -20^{\circ}\text{C}$. Avoid repeated freeze-thaw cycles. Samples may require dilution with Diluent 1X.

REAGENT PREPARATION

Bring all reagents to room temperature before use.

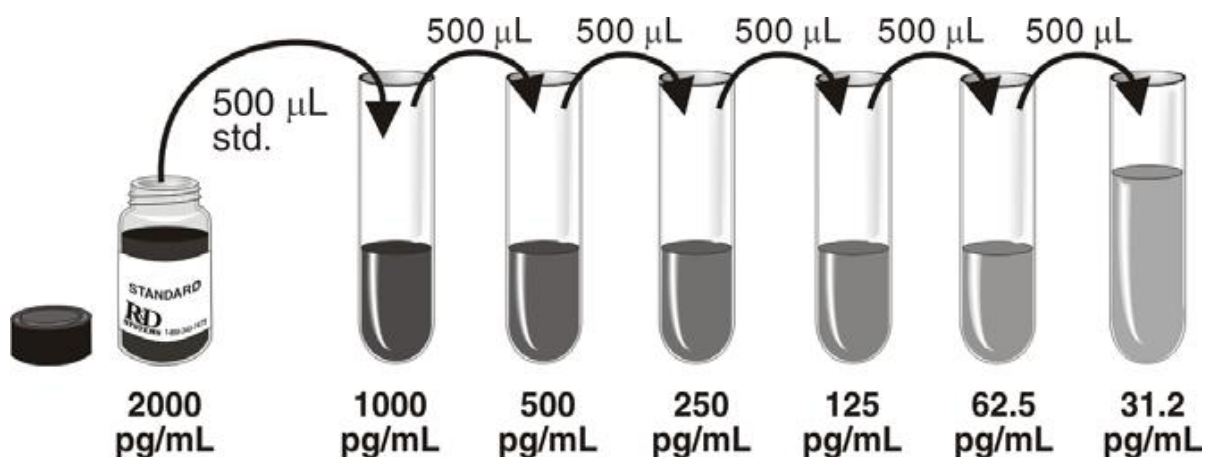
Wash Solution - If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. Dilute 20 mL of Wash Solution Concentrate into deionized or distilled water to prepare 500 mL of Wash Buffer.

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.

Diluent 1X – Add 20 mL of Diluent Concentrate 5X into 80 mL of deionized or distilled water to prepare 100 mL of Diluent 1X.

IL-2 Standard - Reconstitute the IL-2 Standard with 5 mL of Diluent 1X. 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.

Pipette 500 μ L of Diluent 1X 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 Diluent 1X serves as the zero standard (0 pg/mL).



ASSAY PROCEDURE

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 μ L 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 μ L) 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 μ L 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 μ L of Substrate Solution to each well. Incubate for 30 minutes at room temperature. **Protect from light.**
8. Add 50 μ L of Stop Solution 1 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.

CALCULATION OF RESULTS

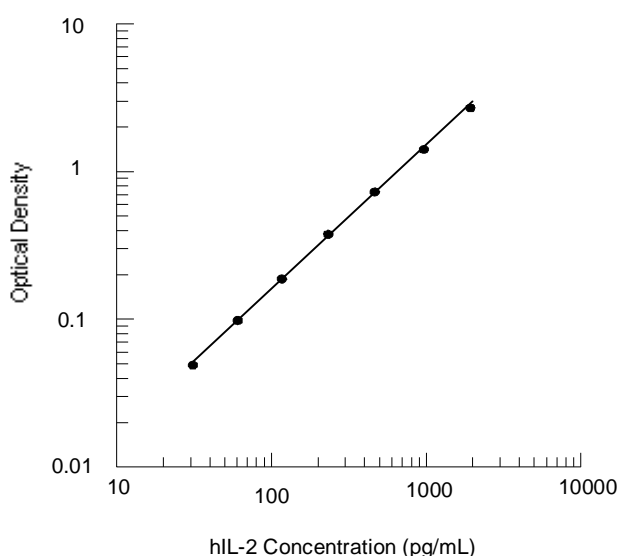
Average the duplicate readings for each standard, control, and sample and subtract the average zero standard optical density.

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.

TYPICAL DATA

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



pg/mL	OD	Average	Corrected
0	0.016	0.017	—
	0.017		
31.2	0.066	0.066	0.049
	0.111		
62.5	0.110	0.111	0.094
	0.213		
125	0.212	0.213	0.196
	0.417		
250	0.431	0.424	0.407
	0.789		
500	0.819	0.804	0.787
	1.562		
1000	1.557	1.560	1.543
	2.741		
2000	2.728	2.735	2.718

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.

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

RECOVERY

The recovery of IL-2 spiked to different levels in cell culture supernates was evaluated. The recovery ranged from 98 - 110% with an average of 106%.

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.

CALIBRATION

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

LINEARITY

To assess the linearity of the assay, four cell culture media samples were spiked with high concentrations of IL-2 and diluted with Diluent 1X 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

SAMPLE VALUES

Human peripheral blood lymphocytes 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.

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:

IL-2 sR α
IL-2 R β
IL-2 R γ
IL-4

Recombinant mouse:

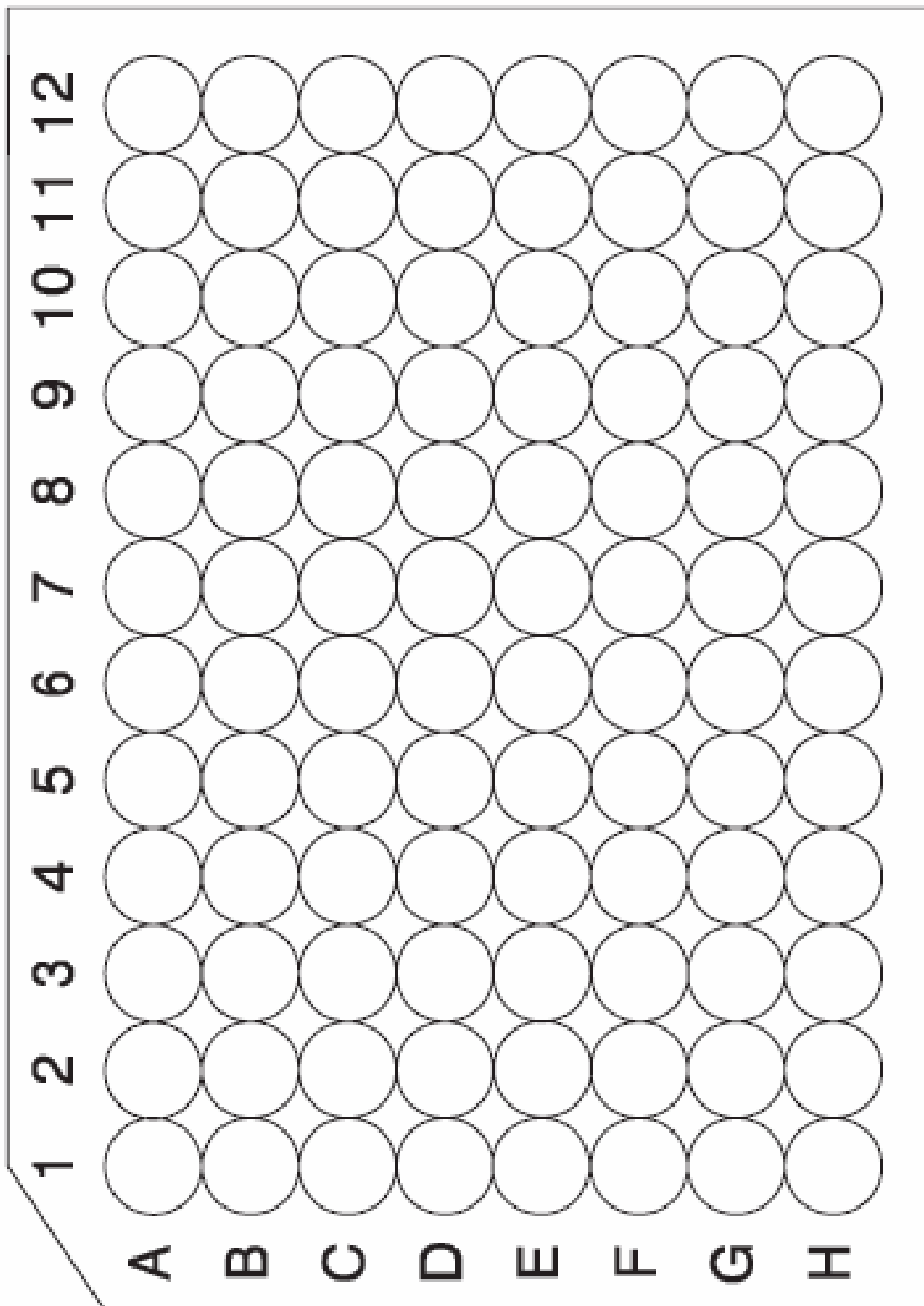
IL-2
IL-4

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. Tadatsugu, 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.



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Valukine™ ELISA 试剂盒

人IL-2 免疫检测试剂盒

目录号：VAL110

适用于定量测定细胞培养上清液中人白介素2（IL-2）的含量

本试剂盒包含足够的试剂以用于一块 96 孔微孔板的 *ELISA* 实验
使用前请仔细阅读产品说明书

科研专用，不可用于临床诊断

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生产:

R&D Systems, Inc.

614 McKinley Place NE

Minneapolis, MN 55413

United States of America

销售和技术支持:

R&D Systems China Co. Ltd.

中国, 上海市, 长宁区

延安西路726号24楼A1座

邮编: 200050

电话: +86 (21) 52380373

传真: +86 (21) 52371001

电邮: info@RnDSystemsChina.com.cn

简介

人白细胞介素 2 (IL-2)，又称为 T 细胞生长因子 (TCGF)，是一个分子量为 15-18 kDa 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/ γ_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)。

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)，溶液颜色逐渐变为蓝色，加入终止液 1 溶液变黄并且停止变化。用酶标仪测定吸光度。

检测局限

- w 仅供科研使用，不可用于体外诊断；
- w 试剂盒请在有效期内使用；
- w 不同试剂盒及不同批号试剂盒的组分不能混用；
- w 样本值若大于标准曲线的最高值，应将样本用稀释剂 (1 \times) 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；
- w 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。

试剂盒组成

包被抗体的 96 孔聚苯乙烯微孔板 / IL-2 Microplate, 8 孔 x12 条
酶标检测抗体 / IL-2 Conjugate, 21ml/瓶, 1 瓶
标准品 (冻干) / IL-2 Standard, 10 ng/瓶, 1 瓶
浓缩稀释剂(5×) / Diluent Concentrate 5X, 21ml/瓶, 1 瓶
浓缩洗涤液(25×) / Wash Solution Concentrate 25X, 21ml/瓶, 1 瓶
显色剂 A / Substrate A, 12.5ml/瓶, 1 瓶
显色剂 B / Substrate B, 12.5ml/瓶, 1 瓶
终止液 1 / Stop Solution 1, 6ml/瓶, 1 瓶
封板胶纸 / Plate Covers, 3 张

试剂盒储存

未开封试剂盒	2 - 8° C 储存; 请在试剂盒有效期内使用	
已打开, 稀释或重溶的试剂	洗涤液(1×)	2 - 8° C 储存, 30 天*
	终止液 1	
	稀释剂(1×)	
	酶标检测抗体	
	显色剂 A	
	显色剂 B	
	标准品	分装, -20° C 以下冰箱储存 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° C 储存, 30 天*	

*必须在试剂盒有效期内

实验所需自备试验器材

- w 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)
- w 高精度加液器及一次性吸头
- w 蒸馏水或去离子水
- w 洗瓶(喷瓶)、多通道洗板器或自动洗板机
- w 500mL 量筒

注意事项

试剂盒中的终止液1是酸性溶液, 使用时请做好眼睛、手、面部及衣服的保护。

样本收集及储存

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电冰箱内, 避免反复冻融。样本可能需要用稀释剂(1×)稀释。

检测前准备工作

使用前请将所有试剂放置于室温

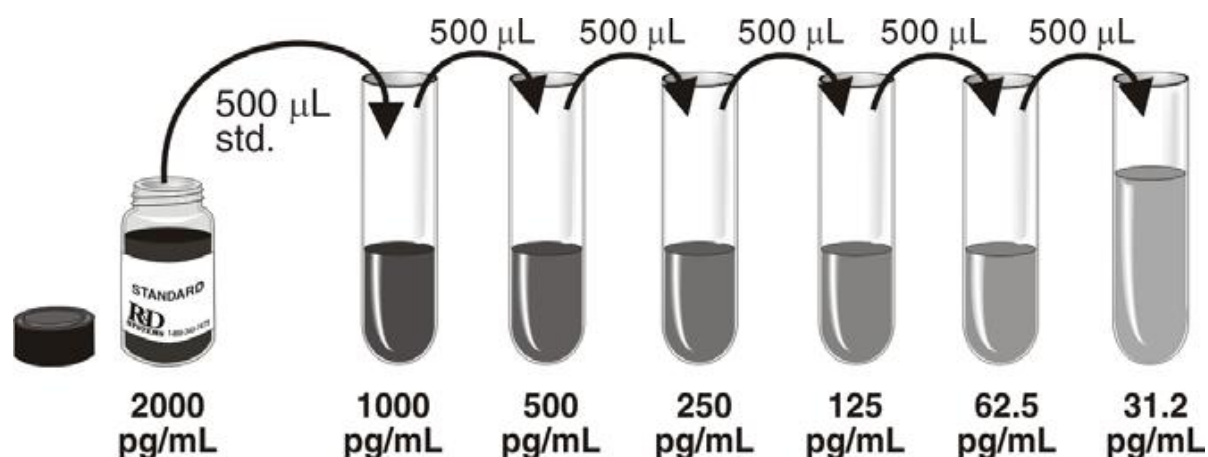
洗涤液: 从冰箱中取出的浓缩洗涤液可能有结晶, 属于正常现象; 放置室温, 轻摇混匀, 待结晶完全溶解后再配制洗涤液。可将20mL浓缩洗涤液用蒸馏水或去离子水稀释配置成500mL工作浓度的洗涤液。未用完的放回4°C。

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分钟准备, 仅供当日使用; 每孔需100 μ L。

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

标准品: 用5.0ml稀释剂(1 \times)重溶冻干标准品, 得到浓度为2000pg/mL标准品母液。轻轻震荡至少15分钟, 其充分溶解。

在每个稀释管中加入500 μ L稀释剂(1 \times)。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 (2000pg/mL), 稀释剂(1 \times)可用作标准曲线零点 (0pg/mL)。



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 μ L。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 μ L，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，在吸水纸上拍干所有残留液体。
5. 在每个微孔内加入200 μ L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 μ L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 μ L终止液1，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1后30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。

计算结果

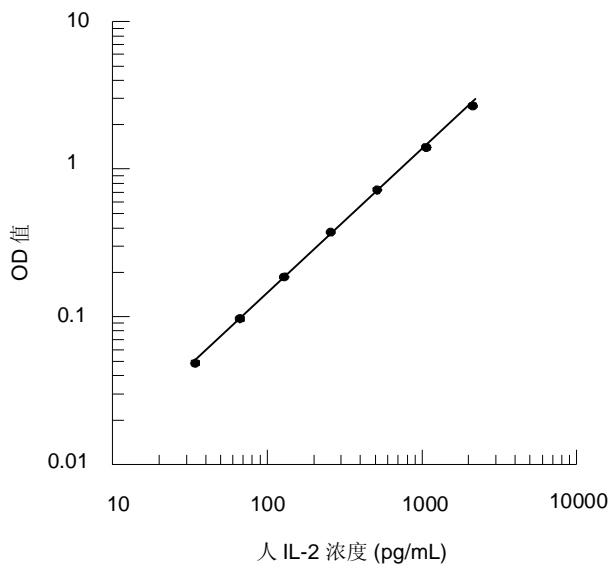
每一个标准品及样本的复孔校正吸光值($OD_{450}-OD_{540}/OD_{570}$)取平均值，再减去标准曲线零点的OD值。

利用酶标仪携带的软件，绘制一个4参数（4-PL）线性标准曲线，曲线横坐标为标准曲线点的人IL-2浓度值，纵坐标为标准曲线点的OD平均值。通过样本的OD值，可从标准曲线上得到样本中人IL-2的浓度。

若样本经过稀释，计算浓度时应乘以稀释倍数。

常见结果

提供的标准曲线数据仅供参考，应根据同次试验所绘标准曲线计算样本含量。



pg/mL	OD	Average	Corrected
0	0.016 0.017 0.066	0.017	—
31.2	0.066 0.111	0.066	0.049
62.5	0.110 0.213	0.111	0.094
125	0.212 0.417	0.213	0.196
250	0.431 0.789	0.424	0.407
500	0.819 1.562	0.804	0.787
1000	1.557 2.741	1.560	1.543
2000	2.728	2.735	2.718

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。

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

回收率

在细胞培养基样本中掺入不同水平的人IL-2，测定其回收率。回收率范围在98 - 110%，平均回收率在106%。

灵敏度

人IL-2的最低可测值一般小于15.6 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。

校正

此ELISA试剂盒经由R&D Systems生产的大肠杆菌表达的高纯重组人IL-2蛋白所校正。

线性

4个不同的样本中掺入高浓度的人IL-2，然后用稀释剂(1×)将样本稀释到检测范围内，测定其线性。

稀释倍数	平均值/期待值 (%)	范围 (%)
1:2	104	101 - 107
1:4	106	101 - 110
1:8	110	101 - 115
1:16	112	99 - 122

样本预值

人的外周血单核细胞培养于含有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 μM β-巯基乙醇、100 U/mL 青霉素，100 μg/mL硫酸链霉素，另加10 μg/mL PHA 刺激细胞，培养24小时。取细胞培养上清液测定IL-2含量，结果为2172 pg/mL。

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×)配置成100 ng/mL的浓度来检测与人IL-2的交叉反应。将干扰因子掺入中间浓度重组人IL-2标准品中，使其浓度到达100 ng/mL，以此来检测对人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：

IL-2 sRα
IL-2 Rβ
IL-2 Rγ
IL-4

重组小鼠蛋白：

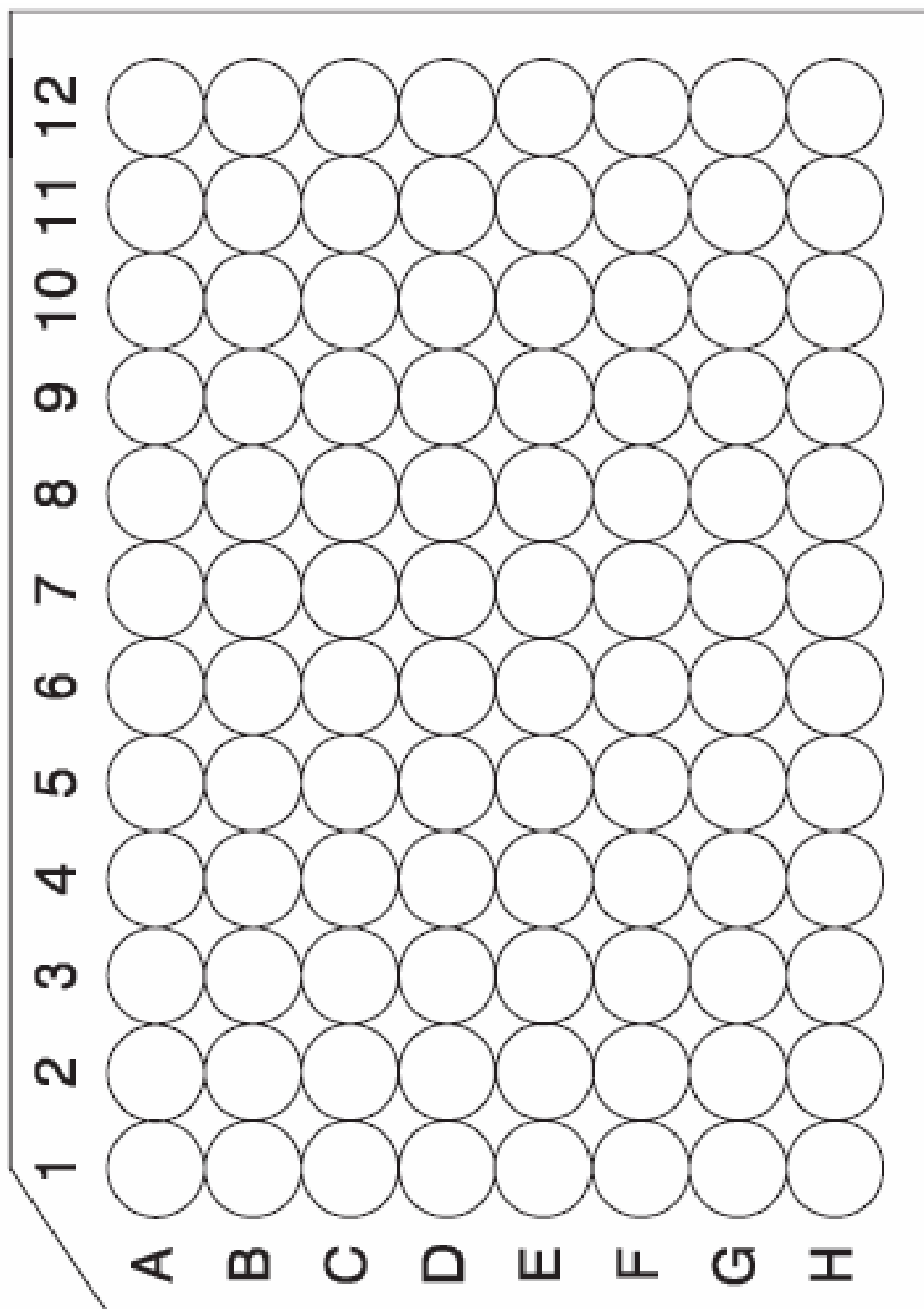
IL-2
IL-4

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. Tadatsugu, 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



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