PRODUCT INFORMATION & MANUAL

Human IL-12 p70 Valukine™ ELISA

VAL115

For the quantitative determination of natural and recombinant human Interleukin (IL)-12 P70 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

Version201904.1

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I. BACKGROUND

Interleukin 12p70 (IL-12), also known as natural killer cell stimulatory factor (NKSF) or cytotoxic lymphocyte maturation factor (CLMF), is a heterodimeric pleiotropic cytokine made up of a 40 kDa (p40) subunit and a 35 kDa (p35) subunit (1,2-4). Human p40 will circulate either as a monomer, homodimer, or in a complex bound to either p35, forming IL-12, or to p19, forming IL-23 (2-4, 5). Both the homodimeric p40, and IL-23 can bind to the IL-12 R, serving as nonsignaling antagonists (2, 6, 7). Alternatively, the p40 homodimer may also bind to Rβ1, activating microglia and macrophages (3, 8). The IL-12 p40 subunit is shared by IL-23, another heterodimeric cytokine that has biological activities similar to, as well as distinct from, IL-12. Cells known to produce IL-12 include macrophages and dendritic cells (9), monocytes (10), Langerhans cells (11), neutrophils (12), keratinocytes (13), plasmacytoid dendritic cells (14), microglia (4), $CD8^+$ DC (mouse cells only) (15) and non-germinal center ($CD38^-CD44^+$) B cells (human cells only) (2, 16). Functionally, IL-12 has been shown to both enhance cytotoxic activity and induce interferon gamma (IFN-γ) production in NK cells, T cells and dendritic epidermal T cells (2, 17-19). IL-12 has also been reported to induce IFN-γ production in macrophages (29). IL-12, in conjunction with the other IL-12 family members IL-23 and IL-27, promotes the development of a $CD4^+$ Th1 immune response (3, 4, 20). In response to infection, IL-27 is released initially, promoting a Th0 to Th0/1 transition. IL-12 production follows, generating Th1 effector cells. In combination with IL-18, IL-12 creates Th1 memory cells out of effector cells, and these cells are later activated by IL-23 (3).While mouse IL-12 is active on both human and mouse cells, human IL-12 is not active on mouse cells.

II. OVERVIEW

A. PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for IL-12 p70 has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any IL-12 p70 present is bound by the immobilized antibody. After washing away any unbound substances, a biotin-linked detect antibody specific for IL-12 p70 are pipetted into the wells. After washing away any unbound substances, streptavidin-HRP is added. 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-12 p70 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 Calibrator 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)	49.9	256	1333.2	53.4	282.9	1484.9
Standard Deviation	2.9	11.6	79.2	4.4	23.4	135.5
CV%	5.7	4.5	5.9	8.2	8.3	9.1

B. RECOVERY

The recovery of human IL-12 p70 spiked to three levels throughout the range of the assay in cell culture media was evaluated. The recovery ranged from 116-119% with an average of 117.6%.

The recovery of human IL-12 p70 spiked to three levels throughout the range of the assay in human serum was evaluated. The recovery ranged from 97.9-105.9% with an average of 101.3%.

SENSITIVITY

The minimum detectable dose (MDD) of IL-12 p70 is typically less than 0.7 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.

C. CALIBRATION

This immunoassay is calibrated against a highly purified *Sf* 21-expressed recombinant human IL-12 p70 produced at R&D Systems®.

D. LINEARITY

To assess the linearity of the assay, different samples were spiked with high concentrations of IL-12 p70 and diluted with Calibrator Diluent to produce samples with values within the dynamic range of the assay.

Dilution	Average % of Expected	Range (%)
1:2	91.8	83.8-98.3
1:4	94.6	81.9-104.4
1:8	100.9	86.6-111.7
1:16	106.9	86.3-117.7

E. SAMPLE VALUES

Serum - Forty samples from apparently healthy volunteers were evaluated for the presence of IL-12 p70 in this assay. No medical histories were available for the donors used in this study. All samples measured less than the lowest human IL-12 p70 standard, 31.3 pg/mL.

F. SPECIFICITY

This assay recognizes both natural and recombinant human IL-12 p70. The following factors prepared at 50 ng/mL were assayed and exhibited no cross-reactivity or interference.

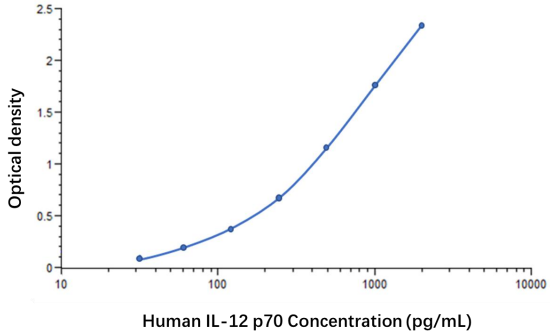
Recombinant human:	**Recombinant mouse:**
IL-12 p35	IL-12 p70
IL-12/IL-23 p40	
IL-23	

IV. EXPERIMENT

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 / SERUM



pg/mL	O.D.		Average	Corrected
0	0.115	0.116	0.116	——
31.3	0.22	0.214	0.217	0.101
62.5	0.315	0.296	0.305	0.189
125	0.496	0.498	0.497	0.381
250	0.812	0.818	0.815	0.699
500	1.278	1.267	1.272	1.156
1000	1.893	1.885	1.889	1.773
2000	2.468	2.439	2.453	2.337

V. KIT COMPONENTS AND STORAGE

A. MATERIALS PROVIDED

Parts	Description	Size
Human IL-12 p70 Microplate	96 well polystyrene microplate (12 strips of 8 wells) coated with a mouse monoclonal antibody against human IL-12 p70	1 plate
Human IL-12 p70 Standard	recombinant human IL-12 p70 in a buffered protein base; lyophilized. Refer to the vial label for reconstitution volume.	2 vials
Human IL-12 p70 detection antibody	Biotinylated IL-12 p70 polyclonal antibody， lyophilized. Refer to the vial label for reconstitution volume.	1 vial
Calibrator Diluent (2×)	Concentrated buffered diluent used to dilute standard and samples .	1 vial
Streptavidin-HRP B (40×)	40× Streptavidin conjugated to horseradish peroxidase	1 vial
Reagent Diluent (10×)	a 10× concentrated buffered protein base used to dilute detection antibody and HRP.	1 vial
Normal goat serum	Diluted, heat-inactive normal goat serum	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 Sealers	adhesive strip	3 strips
Standard Preparation Bottles	Used for standard reconstitution	2 bottles

B. STORAGE

<table>
<tr><td>Unopened Kit</td><td colspan="2">Store at 2-8℃. Do not use past kit expiration date.</td></tr>
<tr><td rowspan="10">Opened/
Reconstituted
Reagents</td><td>Streptavidin-HRP B</td><td rowspan="6">May be stored for up to 1 month at 2-8 °C.*</td></tr>
<tr><td>Normal goat serum</td></tr>
<tr><td>Diluted Wash Solution</td></tr>
<tr><td>Unmixed Color Reagent A</td></tr>
<tr><td>Unmixed Color Reagent B</td></tr>
<tr><td>Stop Solution</td></tr>
<tr><td>Calibrator Diluent</td><td>Prepare fresh for each assay.</td></tr>
<tr><td>Standard</td><td>Aliquot and store for up to 1 month at -20℃ in a manual defrost freezer. *</td></tr>
<tr><td>Detection antibody</td><td>May be stored for up to 1 month at 2-8 °C.*
Use and discard diluted Reagent Diluent. Prepare fresh for each assay.</td></tr>
<tr><td>Reagent diluent</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℃.*</td></tr>
<tr><td>Microplate Wells</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.
- Test tubes for dilution of standards.
- 100mL and 500 mL graduated cylinder.

D. PRECAUTION

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

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.

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. REAGENT PREPARATION

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

Wash Buffer - 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.

Reagent Diluent (1×) - Add 5 mL of Reagent Diluent (10×) into 45 mL of deionized or distilled water to prepare 50 mL of Reagent Diluent (1×).

Calibrator Diluent (1×) – use deionized or distilled water to prepare Calibrator Diluent 1×.

Detection Antibody- Reconstitution Volume refer to vial label with Reagent Diluent (1×)**.** Aliquot and store if needed**.** Dilute stock solution in Reagent Diluent (1×) with 2% heat inactivated normal goat serum (NGS) (200 µL NGS per 10 mL solution) to the working concentration of 100 ng/mL. Prepare at least 15 minutes prior to use.

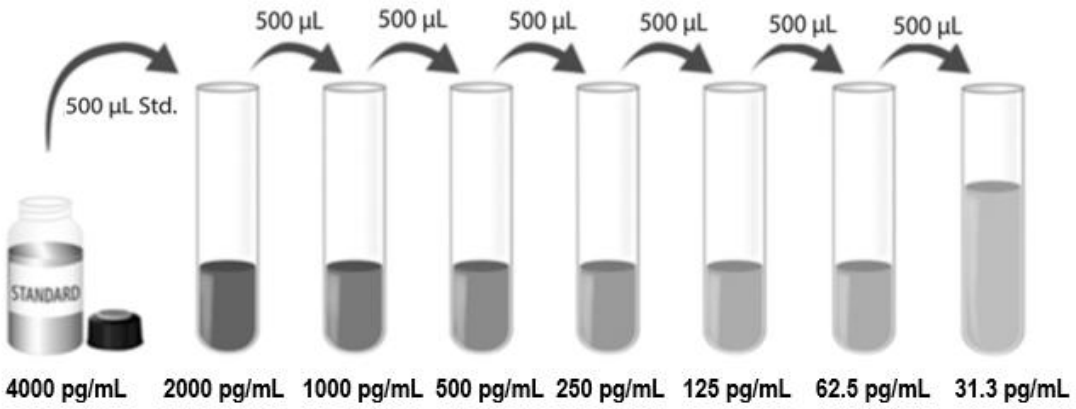
Streptavidin-HRP B- (1×) **-** Dilute to the working concentration specified on the vial label with Reagent Diluent (1×).

Substrate Solution - Color Reagent A and Color Reagent B should be mixed together in equal volumes within 15 minutes of use. Protect from light. 100 µL of the resultant mixture is required per well.

IL-12 p70 Standard – **Refer to the vial label for the reconstitution volume* using Calibrator Diluent.** This reconstitution produces a stock solution of 4000 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 µL of Calibrator Diluent 1× into each tube. Use the stock solution to produce a dilution series (below). Mix each tube thoroughly before the next transfer. The 2000 pg/mL standard serves as the high standard. The **Calibrator Diluent 1×** serves as the zero standard (0 pg/mL).



C. TECHNICAL HINTS

- When mixing or reconstituting protein solutions, always avoid foaming.
- To avoid cross-contamination, change pipette tips between additions of each standard level, between sample additions, and between reagent additions. Also, use separate reservoirs for each reagent.
- It is recommended that the samples be pipetted within 15 minutes.
- To ensure accurate results, proper adhesion of plate sealers during incubation steps is necessary.
- 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, or sample per well. Cover with the adhesive strip provided. Incubate for 2 hours at room temperature. A plate layout is provided for a record of standards and samples assayed.
4. Aspirate each well and wash, repeating the process two times for a total of three 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 100 µL of the Detection Antibody, diluted in Reagent Diluent with NGS, to each well. Cover with a new adhesive strip and incubate 2 hours at room temperature.
6. Repeat the aspiration/wash as in step 4.
7. Add 100 µL of the working dilution of Streptavidin-HRP B to each well. Cover the plate and incubate for 20 minutes at room temperature. Avoid placing the plate in direct light.
8. Repeat the aspiration/wash as in step 4.
9. Add 100 µL of Substrate Solution to each well. Incubate for 20 minutes at room temperature. Avoid placing the plate in direct light.
10. Add 50 µL of Stop Solution to each well. Gently tap the plate to ensure thorough mixing.
11. Determine the optical density of each well immediately, 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.
12. **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 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-12 p70 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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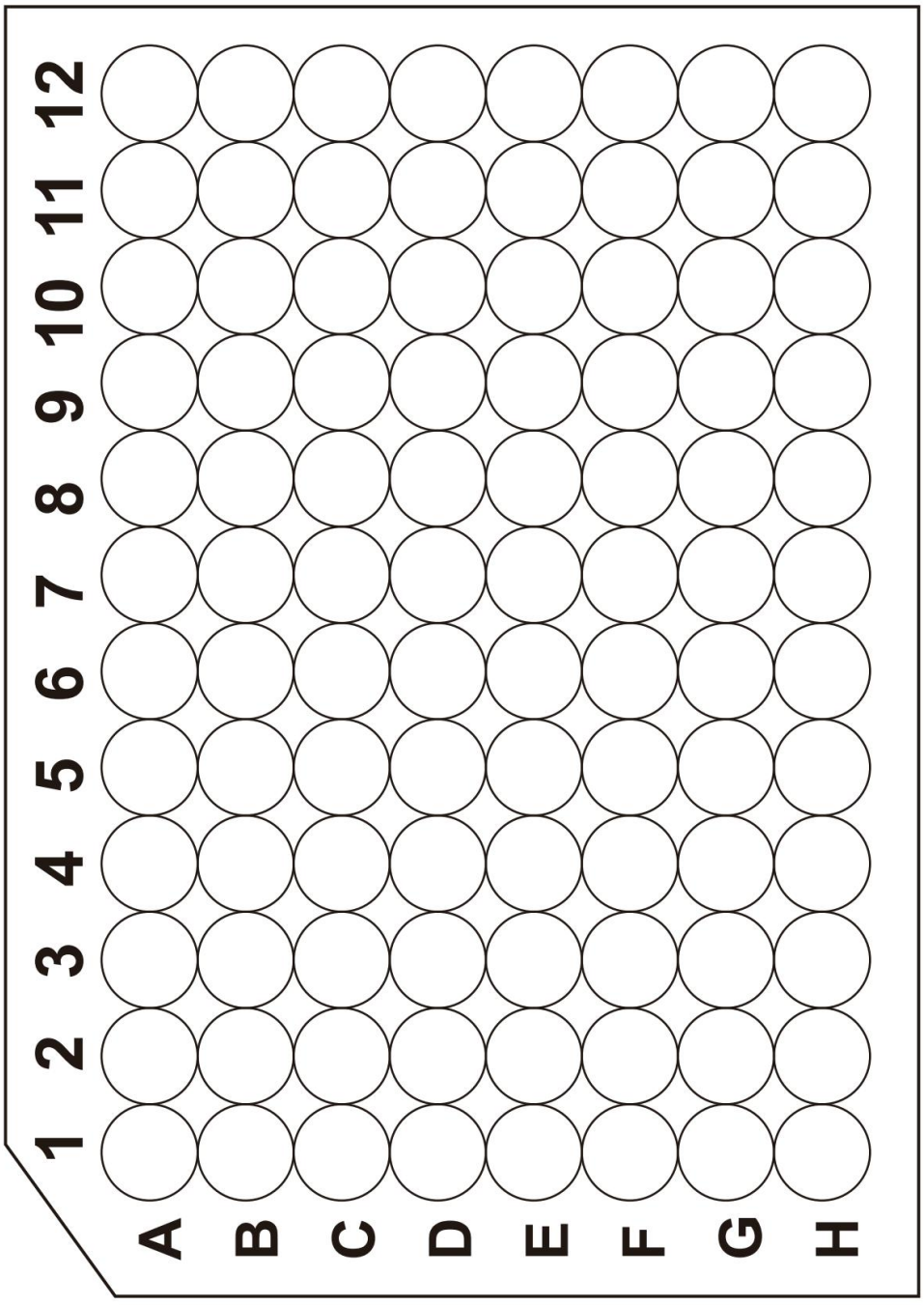
PLATE LAYOUT

Use this plate layout to record standards and samples assayed.



产品信息及操作手册

人 IL-12 p70 Valukine™ ELISA 试剂盒

目录号：VAL115

适用于定量检测天然和重组人白介素 12 P70（IL-12P70）的浓度

科研专用，不可用于临床诊断

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目录

I. 背景

白细胞介素 12p70（IL-12），又称自然杀伤细胞刺激因子（NKSF）或细胞毒性淋巴细胞成熟因子（CLMF），是一种由 40 kDa（p40）亚基和 35 kDa（p35）亚基组成的异二聚多效细胞因子（1,2-4）。人 p40 可作为单体、均二聚体或与 p35 结合形成 IL-12 或与 p19 结合形成 IL-23 的复合物循环(2- 4,5)。同源二聚体 p40 和 IL-23 均可与 IL-12R 结合，作为非信号拮抗剂（2,6,7）。或者,p40 为也可以绑定到 Rβ1,激活小胶质细胞和巨噬细胞（3,8）。IL-12 p40 亚基与另一种异二聚细胞因子 IL-23 共享，与 IL-12 相似且不同的生物活性。已知产生 IL-12 的细胞包括巨噬细胞和树突状细胞（9）、单核细胞（10）、朗格汉斯细胞（11）、中性粒细胞（12）、角质形成细胞（13）、浆细胞样树突状细胞（14）、小胶质细胞（4）、$CD8^+$ DC（仅小鼠细胞）（15）和非生发中心（$CD38^-CD44^+$）B 细胞（仅人类细胞）（2,16）。功能性 IL-12 显示增强细胞毒活性和诱导 NK 细胞、T 细胞和树突状表皮 T 细胞（2, 17-19）内干扰素γ（IFN-γ）的产生。IL12 也被报道可由巨噬细胞诱导 IFN-γ生产（29）。IL-12 与 IL-12 家族其它成员 IL-23 和 IL-27 共同促进 $CD4^+$ Th1 免疫反应的发展(3,4,20)。作为对感染的反应，IL-27 首先被释放，促进 Th0 到 Th0/1 的转变。随后产生 IL-12，产生 Th1 效应细胞。IL-12 与 IL-18 联合，从效应细胞中产生 Th1 记忆细胞，这些细胞随后被 IL-23 激活（3）。虽然小鼠 IL-12 对人和小鼠细胞都有活性，但人 IL-12 对小鼠细胞没有活性。

II. 概述

A. 检测原理

本实验采用双抗体夹心 ELISA 法。抗人 IL-12 p70 单抗包被于微孔板上，经过孵育，样品和标准品中的 IL-12 p70 会与固定在板上的抗体结合，游离的成分被洗去；接着加入生物素化的抗人 IL-12 p70 多抗进行孵育，洗涤去除未结合的物质后，加入辣根过氧化物酶标记的链霉亲和素(streptavidin-HRP)孵育。洗涤后，加入显色底物，避光显色。溶液颜色与结合的目标蛋白成正比；随后加入终止液，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，应将样本用标准品稀释剂（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，在不同板中分别检测 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）	49.9	256	1333.2	53.4	282.9	1484.9
标准差	2.9	11.6	79.2	4.4	23.4	135.5
CV%	5.7	4.5	5.9	8.2	8.3	9.1

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-12 p70，测定其回收率。回收率范围在 116-119%，平均回收率在 117.6%。

在人血清样本中掺入检测范围内不同水平的人 IL-12 p70，测定其回收率。回收率范围在 97.9-105.9%，平均回收率在 101.3%。

C. 灵敏度

人 IL-12 p70 的最低可测剂量（MDD）一般小于 0.7 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

此 ELISA 试剂盒经由 R&D Systems®生产的 *Sf* 21 表达的高纯度重组人 IL-12 p70 蛋白所校正。

E. 线性

不同的样本中掺入高浓度的人 IL-12 p70，然后用标准品稀释剂（1×）将样本稀释到检测范围内，测定其线性。

稀释倍数	平均期待值（%）	范围（%）
1:2	91.8	83.8-98.3
1:4	94.6	81.9-104.4
1:8	100.9	86.6-111.7
1:16	106.9	86.3-117.7

F. 样本值

血清- 使用本试剂盒检测了 40 份来自健康志愿者的样本中 IL-12p70 的水平。这些样本供应者供者没有病史。所有样本均低于人 IL-12 p70 最低标准 31.3 pg/mL。

G. 特异性

此 ELISA 法可检测天然及重组人 IL-12 p70 蛋白。对制备的 50 ng/mL 的下列因素进行了测定，无交叉反应或干扰。

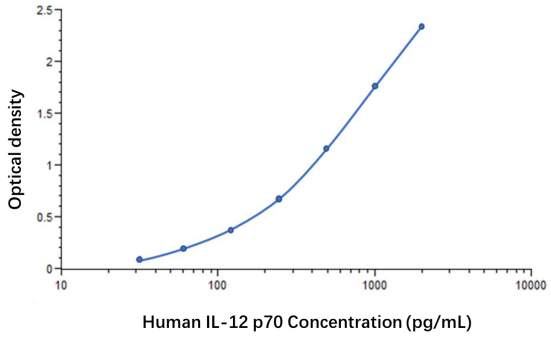
重组人：	重组小鼠：
IL-12 p35	IL-12 p70
IL-12/IL-23 p40	
IL-23	

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。

细胞培养上清/血清



pg/mL	O.D.		Average	Corrected
0	0.115	0.116	0.116	——
31.3	0.22	0.214	0.217	0.101
62.5	0.315	0.296	0.305	0.189
125	0.496	0.498	0.497	0.381
250	0.812	0.818	0.815	0.699
500	1.278	1.267	1.272	1.156
1000	1.893	1.885	1.889	1.773
2000	2.468	2.439	2.453	2.337

V. 试剂盒组成及储存

A. 试剂盒组成

组成	描述	规格
Human IL-12 p70 Microplate	包被小鼠抗人 IL-12 p70 单抗的 96 孔聚苯乙烯板，8 孔×12 条	1 块板
Human IL-12 p70 Standard	标准品（冻干粉）,参考瓶标签进行重溶。	2 瓶
Human IL-12 p70 detection antibody	生物素化的 IL-12 p70 检测抗体，冻干粉，参考瓶标签进行重溶。	1 瓶
Calibrator Diluent (2×）	浓缩的样品和标准品稀释剂（2×）	1 瓶
Streptavidin-HRP B (40×)	40×浓缩链霉亲和素标记的 HRP	1 瓶
Reagent Diluent (10×）	浓缩的试剂稀释液（10 ×）	1 瓶
Normal goat serum	稀释的热灭活正常山羊血清	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 Sealers	封板膜	3 张
Standard Preparation Bottles	用于标准品重溶	2 瓶

B. 试剂盒储存

<table>
<tr><td>未开封试剂盒</td><td colspan="2">2-8° C 储存；请在试剂盒有效期内使用</td></tr>
<tr><td rowspan="10">已打开，稀释或重溶的试剂</td><td>链霉亲和素-HRP</td><td rowspan="7">2-8℃储存，最多 30 天*</td></tr>
<tr><td>山羊血清</td></tr>
<tr><td>洗涤缓冲液（1×）</td></tr>
<tr><td>显色剂 A</td></tr>
<tr><td>显色剂 B</td></tr>
<tr><td>终止液</td></tr>
<tr><td>标准品稀释液</td></tr>
<tr><td>标准品</td><td>使用时新鲜配制。</td></tr>
<tr><td>检测抗体</td><td>储存浓度 2-8℃储存，最多 30 天*</td></tr>
<tr><td>试剂稀释液（10×）</td><td>2-8℃储存，最多 30 天*。稀释后的 1×稀释液请弃去，每次使用新鲜配制的。</td></tr>
<tr><td>包被的微孔板条</td><td>将未用的板条放回带有干燥剂的铝箔袋内，密封；2-8℃储存，最多 30 天*</td></tr>
</table>

*必须在试剂盒有效期内

C. 实验所需自备试剂器材

- 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
- 用于稀释标准品的试管
- 100 mL 及 500 mL 量筒

D. 注意事项

- 试剂盒中的一些组分包含防腐剂，可能引起皮肤过敏反应。避免吸入。
- 试剂盒组分中显色液 B，可能引起皮肤、眼睛和呼吸道刺激或皮肤过敏反应。避免吸入。
- 试剂盒中的终止液是酸性溶液，使用时请做好眼镜、手、面部及衣服的防护。
- 实验穿戴防护衣服、手套、眼睛和脸的保护罩。使用后请彻底洗手。

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。

血清样本: 用血清分离管(SST)分离血清。在1000 g离心15分钟之前，使血样室温凝集30分钟或4°C过夜。吸取血清样本之后即刻检测，或者分装，-20℃以下贮存。避免反复冻融。

B. 检测前准备工作

使用前请将所有试剂放置于室温

洗涤液：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置成 500 mL 工作浓度的洗涤液。未用完的放回 4 ℃。

试剂稀释液（1×）：加 5 mL 试剂稀释液(10×)到 45 mL 蒸馏水或去离子水中来准备 50 mL 的试剂稀释液 (1×)。

标准品稀释剂（1×）：使用蒸馏水或去离子水稀释配置成标准品稀释剂（1×）。

检测抗体：参考瓶子标签的指示用试剂稀释液 (1×)将冻干粉进行重溶。再用添加了 2% 热灭活的正常山羊血清 (NGS) (每 10 毫升溶液加 200 µL NGS) 的试剂稀释液 (1×)稀释至工作浓度 100ng/mL，至少在使用前 15 分钟准备。

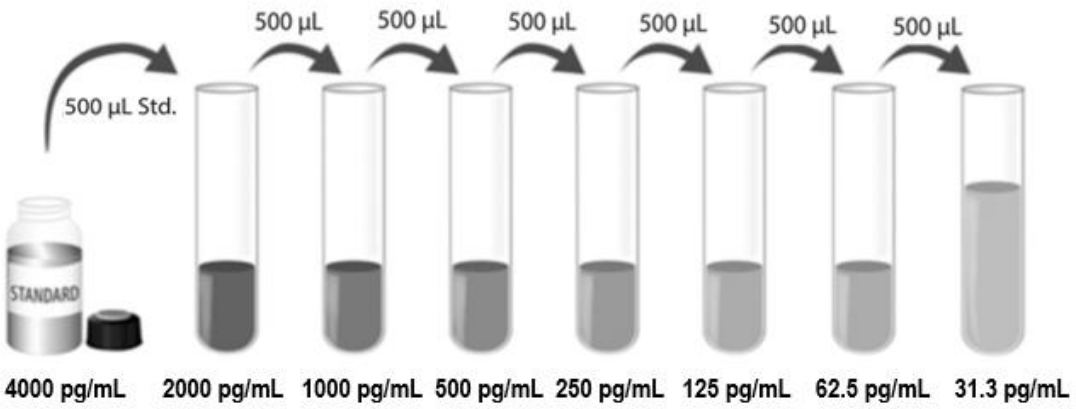
链霉亲和素- HRP B：用试剂稀释液 (1×) 将链霉亲和素- HRP B（40×）稀释至工作浓度-链霉亲和素- HRP B（1×）。

显色剂：按当次试验所需要用量将显色液 A 和显色液 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 100 µL。

标准品：冻干标准品的重溶体积请参考瓶身标签*，用标准品稀释剂 (1×) 重溶，得到 4000 pg/mL 的标准品母液。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 **标准品稀释剂（1×）**。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。2000 pg/mL 管作标准曲线最高点，**标准品稀释剂（1×）**可用作标准品零点（0 pg/mL）。



C. 技术小提示

- 当混合或重溶蛋白液时，尽量避免起沫；
- 为了避免交叉污染，配置不同浓度标准品、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，正确使用封板膜可保证结果的准确性；
- 混合后的显色剂在上板前应为无色，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。

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 100 µL。用封板膜封住反应孔，室温孵育 2 小时。说明书提供了一张 96 孔模板图，可用于记录标准品和试验样本的板内位置；
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 2 次，共洗涤 3 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 100 µL 配置好的检测抗体。用封板膜封住反应孔，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 100 µL 稀释好的链霉亲和素- HRP B 工作液。用封板膜封住反应孔，室温避光孵育 20 分钟；
8. 重复第 4 步洗板操作；
9. 在每个微孔内加入 100 µL 显色剂，室温孵育 20 分钟。**注意避光**；
10. 在每个微孔内加入 50 µL 终止液，请轻拍微孔板，使溶液混合均匀。孔内溶液颜色会从蓝色变为黄色。
11. 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；
12. **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。通过样本的 OD 值，可从标准曲线上得到样本中人 IL-12 p70 的浓度。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。

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