PRODUCT INFORMATION & MANUAL

Human IL-10 Valukine™ ELISA

VAL112

For the quantitative determination of natural and
recombinant human IL-10 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

Version 201910.2

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I. BACKGROUND

Interleukin-10 (IL-10), also known as cytokine synthesis inhibitory factor (CSIF), is the charter member of the IL-10 α-helical cytokine family that also includes IL-19, IL-20, IL-22, IL-24, and IL-26/AK155 (1-3). IL-10 is secreted by many activated hematopoietic cell types as well as hepatic stellate cells, keratinocytes, and placental cytotrophoblasts. Whereas human IL-10 is active on mouse cells, mouse IL-10 does not act on human cells (4, 5). Mature human IL-10 shares 86% amino acid sequence identity with equine IL-10 and 72%-80% with bovine, canine, feline, guinea pig, mouse, ovine, porcine, and rat IL-10. It contains two intrachain disulfide bridges and is expressed as a 36 kDa noncovalently-associated homodimer (4, 6, 7).

IL-10 mediates its biological activities through a heteromeric receptor complex composed of the type II cytokine receptor subunits IL-10 Rα and IL-10 Rβ. IL-10 Rα is a 110 kDa transmembrane glycoprotein that is expressed on lymphocytes, NK cells, macrophages, monocytes, astrocytes, intestinal epithelial cells, cytotrophoblasts, and activated hepatic stellate cells (8-13), while the 75 kDa transmembrane IL-10 Rβ is widely expressed (14, 15). The IL-10 dimer binds to two IL-10 Rα chains, triggering recruitment of two IL-10 Rβ chains (14, 15). IL-10 Rβ does not bind IL-10 directly but is required for signal transduction. IL-10 Rβ also associates with IL-20 Rα, IL-22 Rα1, or IL-28 Rα to form the receptor complexes for IL-22, IL-26, IL-28, and IL-29 (16-18).

The involvement of IL-10 in immunoregulation includes both suppressive and stimulatory effects. It functions as an anti-inflammatory cytokine by inhibiting the expansion and activation of Th1 cells and Th17 cells (19-21) and by promoting the development of M2 macrophages (21). Its expression by immunosuppressive regulatory T cells (Treg) and regulatory B cells is important for Treg proliferation (19). Within a tumor microenvironment, however, IL-10 inhibits the expansion of Treg as well as myeloid-derived suppressor cells (22, 23). IL-10 induces the intratumoral accumulation and activation of $CD8^+$ T cells (24, 25). IL-10 exerts protective effects including limiting tissue damage in arthritic inflammation (19) and promoting muscle regeneration after injury (21), but it also contributes to the persistence of viral infections (26). The levels of IL-10 are elevated in Sjogren's syndrome (saliva), primary CNS lymphoma (cerebrospinal fluid), and ovarian cancer (serum and ascites) (27-29). Its levels are decreased in the serum in patients with recurrent heart attacks or during preeclampsia and also in the seminal fluid of infertile men (30-32).

II. OVERVIEW

A. PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for IL-10 has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any IL-10 present is bound by the immobilized antibody. After washing away any unbound substances, an enzyme-linked polyclonal antibody specific for IL-10 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-10 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 forty separate assays to assess inter-assay precision. Assay were performed by at least three technicians using two lots of components.

	Intra-assay Precision			**Inter-assay Precision**		
Sample	1	2	3	1	2	3
n	20	20	20	40	40	40
Mean (pg/mL)	24.4	79.6	223	23.6	74.2	227
Standard Deviation	1.6	3.9	5.5	1.8	4.9	12.7
CV%	6.6	4.9	2.5	7.9	6.6	5.6

B. RECOVERY

The recovery of human IL-10 spiked to different levels throughout the range of the assay in cell culture media was evaluated. The recovery ranged from 88-110% with an average of 98%.

The recovery of human IL-10 spiked to different levels throughout the range of the assay in human serum was evaluated. The recovery ranged from 77.1-85.2% with an average of 80.2%.

C. SENSITIVITY

The minimum detectable dose (MDD) of IL-10 is typically less than 3.9 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 *Sf21*-expressed recombinant human IL-10 produced at R&D Systems®.

E. LINEARITY

To assess the linearity of the assay, different samples were containing or spiked with high concentrations of IL-10 and diluted with Calibrator Diluent 1× to produce samples with values within the dynamic range of the assay.

Dilution	**Average % of Expected**	**Range (%)**
1:2	98	92 – 108
1:4	96	86 – 109
1:8	94	84 – 104
1:16	90	82 – 98

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. Cells were cultured unstimulated or stimulated with 10 µg/mL PHA for 1, 3 and 5 days. Aliquots of the cell culture supernate was removed and assayed for human IL-10.

Condition	Day 1 (pg/mL)	Day 3 (pg/mL)	Day 5 (pg/mL)
Unstimulated	98	134	127
Stimulated	2756	3563	2255

Serum - Four serum samples were evaluated for the presence of IL-10 in this assay. All samples measured below the lowest standard, 7.8pg/mL.

G. SPECIFICITY

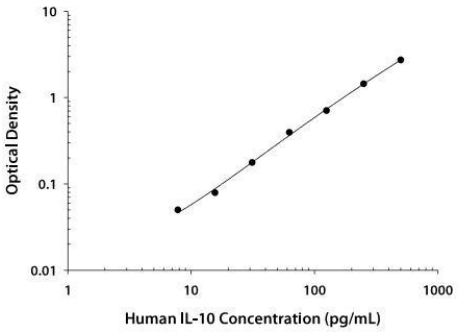
This assay recognizes both natural and recombinant human IL-10. The following factors were prepared at 50 ng/mL in Calibrator Diluent 1x and assayed for cross-reactivity. Preparations of the following factors at 50 ng/mL in a mid-range rhIL-10 control were assayed for interference. No significant cross-reactivity or interference was observed.

Recombinant Human	**Recombinant Mouse**
IL-10 (cytomegalovirus)	IL-10
IL-10 Rα	IL-10 Rα
IL-10 Rβ/Fc Chimera	
Other Recombinants	
Canine IL-10	Porcine IL-10
Equine IL-10	Rat IL-10
Feline IL-10	Viral IL-10 (Epstein-Barr)

IV. EXPERIMENT

EXAMPLE STANDARD

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



(pg/mL)	O.D.	Average	Corrected
0	0.028 0.032	0.030	—
7.8	0.071 0.088	0.080	0.050
15.6	0.108 0.110	0.109	0.079
31.3	0.203 0.211	0.207	0.177
62.5	0.418 0.432	0.425	0.395
125	0.718 0.755	0.737	0.707
250	1.440 1.506	1.473	1.443
500	2.729 2.779	2.754	2.724

V. KIT COMPONENTS AND STORAGE

A. MATERIALS PROVIDED

Parts	Description	Size
IL-10 Microplate	96 well polystyrene microplate (12 strips of 8 wells) coated with a mouse monoclonal antibody against human IL-10	1 plate
IL-10 Conjugate	A solution of monoclonal antibody against IL-10 conjugated to horseradish peroxidase	1 vial
IL-10 Standard	Recombinant human IL-10 in a buffered protein base; lyophilized	1 vial
Calibrator Diluent(5×)	A 5× concentrated buffered protein base	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

<table>
<tr><td>Unopened Kit</td><td colspan="2">Store at 2-8°C. Do not use past kit expiration date.</td></tr>
<tr><td rowspan="8">Opened/ Reconstituted Reagents</td><td>Diluted Wash Solution</td><td rowspan="6">May be stored for up to 1 month at 2-8°C.*</td></tr>
<tr><td>Stop Solution</td></tr>
<tr><td>Calibrator Diluent 1×</td></tr>
<tr><td>Conjugate</td></tr>
<tr><td>Unmixed color reagent A</td></tr>
<tr><td>Unmixed color reagent B</td></tr>
<tr><td>Standard</td><td>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>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.
- Polypropylene tubes for dilution of standards and samples.

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 µL of sample + 100 µL of Calibrator Diluent (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.

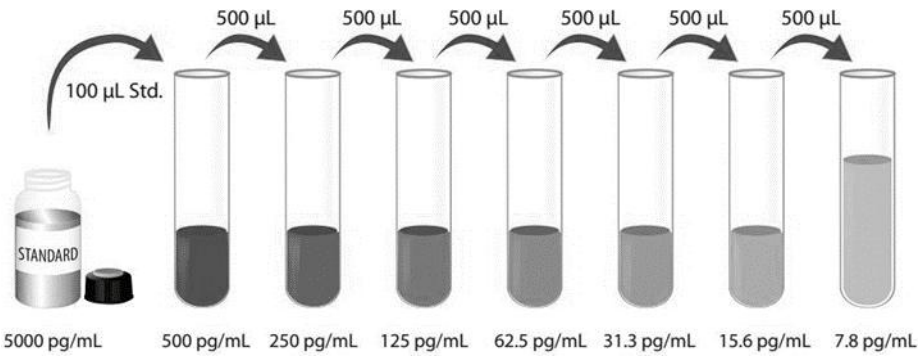
Substrate Solution - Color Reagent A and B should be mixed together in equal volumes within 15 minutes of use. Protect from light. 200 µL of the resultant mixture is required per well.

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-10 Standard - Refer to the vial label for reconstitution volume*. This reconstitution produces a stock solution of 5000 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.

Use polypropylene tubes. Pipette 900 µL of Calibrator Diluent 1x into 500 pg/mL tube. Pipette 500 µL of the appropriate Calibrator Diluent 1x into each tube. Use the stock solution to produce a dilution series (below). Mix each tube thoroughly before the next transfer. The 500 pg/mL standard serves as the high standard. The appropriate Calibrator Diluent 1x 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 200 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-10 Conjugate to each well. Cover with a new adhesive strip. Incubate for 1 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-10 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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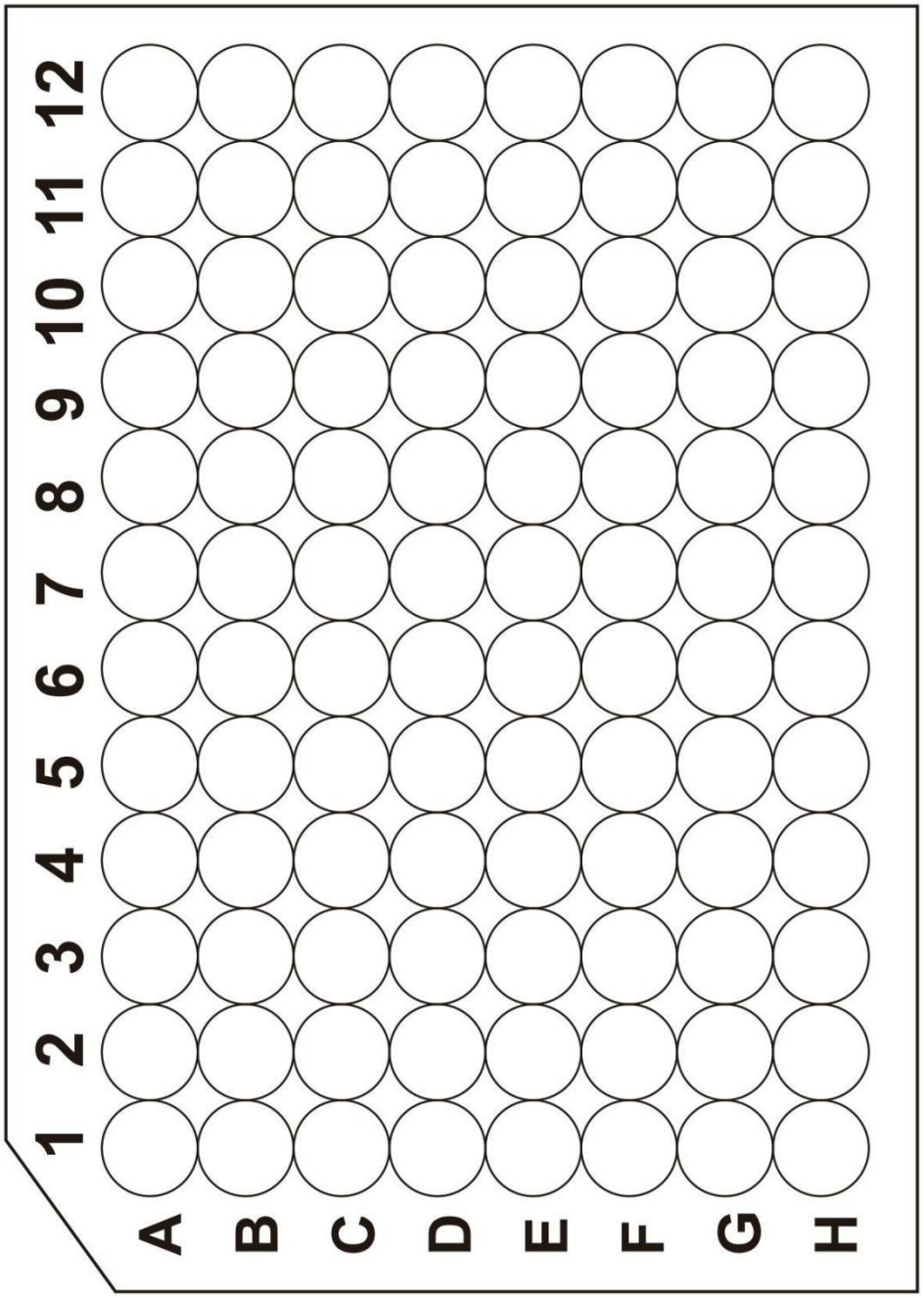
PLATE LAYOUT

Use this plate layout to record standards and samples assayed.



产品信息及操作手册

人 IL-10 Valukine™ ELISA 试剂盒

目录号：VAL112

适用于定量检测天然和重组人白介素 10（IL-10）的含量

科研专用，不可用于临床诊断

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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10（IL-10），又称为细胞生长因子合成抑制因子（CSIF），是 IL-10α螺旋家族的成员，该家族还包括 IL-19、IL-20、IL-22、IL-24 和IL-26/ak155（1-3）。IL-10可由多种活化的造血细胞、肝星状细胞、角质形成细胞和胎盘滋养叶细胞分泌。人类IL-10对小鼠细胞有活性，而小鼠IL-10 不能作用于人的细胞（4, 5）。成熟的人IL-10 与马IL-10有86%的氨基酸序列同源性，与牛、犬、猫、豚鼠、小鼠、绵羊、猪和大鼠IL-10 的同源性在72-80%之间。它包含两个链内二硫键，以非共价结合的同源二聚体形式表达，分子量36kDa （4, 6, 7）。

IL-10 通过由II型细胞因子IL-10受体亚基α和β异源二聚体复合物介导其生物活性。IL-10受体亚基是一个110kDa 的跨膜糖蛋白，主要表达于淋巴细胞、NK 细胞、巨噬细胞、单核细胞、星形胶质细胞、肠上皮细胞、滋养层细胞和活化的肝星状细胞（8-13），而75kDa 的跨膜受体β则广泛表达（14, 15）。IL-10 二聚体结合两个IL-10 Rα链，激发两个IL-10 Rβ链的加入（14, 15）。IL-10 Rβ不直接结合IL-10，但为信号转导所需要。IL-10 Rβ还与IL-20 Rα、IL-22 Rα1及IL-28 Rα形成IL-22、IL-26、IL-28 和IL-29 的受体复合物（16-18）。

IL-10 参与的免疫调节包括抑制作用和刺激作用。它通过抑制 Th1 细胞和Th17 细胞的扩增和活化（19-21），促进M2 型巨噬细胞（21）发挥抗炎作用。免疫调节 T 细胞（Treg）和调节性B 细胞对IL-10 表达的调节对Treg 增殖有重要意义（19）。然而，在肿瘤环境中，IL-10 抑制Treg 及髓源性抑制细胞的增殖（22, 23）。IL-10 诱导CD8 T 细胞在肿瘤中的积累和活化（24, 25）。IL-10 通过限制关节炎的组织损伤（19），促进肌肉损伤后再生（21）发挥保护作用，但它可能导致病毒感染的持久性（26）。IL-10 水平在干燥综合征（唾液）、原发性中枢神经系统淋巴瘤（脑脊液）、卵巢癌（血清和腹水）（27-29）中升高。其水平在心脏病复发或子痫前期患者血清和男性不育患者精液中降低（30-32）。

II. 概述

A. 检测原理

本实验采用双抗体夹心ELISA法。抗人IL-10 单抗包被于微孔板上，样品和标准品中的IL-10 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-10多抗，与未结合的抗体被洗去；加入底物溶液（显色剂），溶液颜色与结合的目标蛋白成正比；加入终止液；用酶标仪测定吸光度。

B. 检测局限

- **仅供科研使用，不可用于体外诊断；**
- 该试剂盒适用于细胞培养上清样本和人血清样本；
- 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；
- 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 40 次，以确定板间精确度。

	板内精确度			板间精确度		
样本	1	2	3	1	2	3
数量	20	20	20	40	40	40
平均值 (pg/mL)	24.4	79.6	223	23.6	74.2	227
标准差	1.6	3.9	5.5	1.8	4.9	12.7
CV%	6.6	4.9	2.5	7.6	6.6	5.6

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-10，测定其回收率。回收率范围在88-110%，平均回收率在 98%。

在人血清样本中掺入不同水平的人IL-10，测定其回收率。回收率范围在77.1-85.2%，平均回收率在 80.2%。

C. 灵敏度

人IL-10 的最低可测剂量（MDD）一般小于3.9 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-10 蛋白所校正。

E. 线性

不同的样本中含有或掺入高浓度的人IL-10，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	98	92– 108
1:4	96	86 – 109
1:8	94	84 – 104
1:16	90	82 – 98

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 μM β-巯基乙醇、100 U/mL 青霉素，100 μg/mL 链霉素，部分另加 10 μg/mL PHA 刺激细胞，在 1、3 和 5 天，取少量未经刺激和刺激后的细胞上清液测定IL-10 含量，结果见下表。

条件	第 1 天 (pg/mL)	第 3 天 (pg/mL)	第 5 天(pg/mL)
未刺激	98	134	127
刺激	2756	3563	2255

血清样本 - 使用本试剂盒检测了4 份人血清样本中 IL-10 的水平。4 份样本的检测值均低于最低标准品，7.8 pg/mL。

G. 特异性

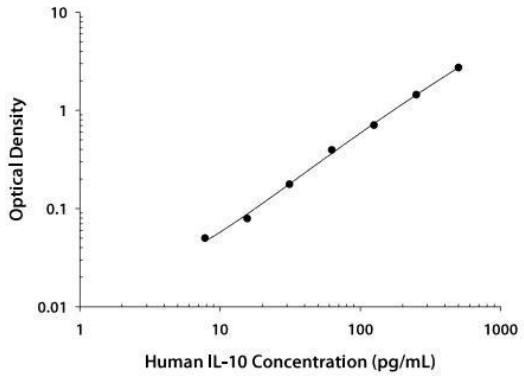
此ELISA法可检测天然及重组人IL-10 蛋白。将以下因子用稀释剂（1×）配置成 50 ng/mL 的浓度来检测与人IL-10的交叉反应。将 50 ng/mL 的干扰因子掺入中间范围的重组人IL-10 对照品中，来检测对人IL-10的干扰。没有观察到明显的交叉反应或干扰。

重组人蛋白	**重组小鼠蛋白**
IL-10 (cytomegalovirus)	IL-10
IL-10 Rα	IL-10 Rα
IL-10 Rβ/Fc Chimera	
其他重组蛋白	
犬 IL-10	猪 IL-10
马IL-10	大鼠 IL-10
猫 IL-10	Viral IL-10 (Epstein-Barr)

IV. 实验标准

标准曲线实例

该标准曲线数据仅供参考，每次实验应绘制其对应的标准曲线。



(pg/mL)	O.D.	Average	Corrected
0	0.028 0.032	0.030	—
7.8	0.071 0.088	0.080	0.050
15.6	0.108 0.110	0.109	0.079
31.3	0.203 0.211	0.207	0.177
62.5	0.418 0.432	0.425	0.395
125	0.718 0.755	0.737	0.707
250	1.440 1.506	1.473	1.443
500	2.729 2.779	2.754	2.724

V. 试剂盒组成及储存

A. 试剂盒组成

组成	描述	规格
IL-10 Microplate	包被抗体的 96 孔聚苯乙烯板，8 孔×12 条	1 块板
IL-10 Conjugate	酶标检测IL-10 抗体	1 瓶
IL-10 Standard	标准品（冻干粉）	1 瓶
Calibrator Diluent（5×）	浓缩稀释剂（5×）	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

B. 试剂盒储存

<table>
<tr><td>未开封试剂盒</td><td colspan="2">2-8℃储存；请在试剂盒有效期内使用</td></tr>
<tr><td rowspan="8">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>酶标检测抗体</td></tr>
<tr><td>显色剂 A</td></tr>
<tr><td>显色剂 B</td></tr>
<tr><td>标准品</td><td>分装，-20℃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

C. 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
- 使用聚丙烯试管稀释标准品和样品
- 洗瓶（喷瓶）、多通道洗板器或自动洗板机
- 500 mL 量筒

D. 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1000 x 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℃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（1×）2倍稀释后进行检测，即100 µL 血清+100 µL稀释剂（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置成500mL 工作浓缩的洗涤液。未用完的放回 4℃。

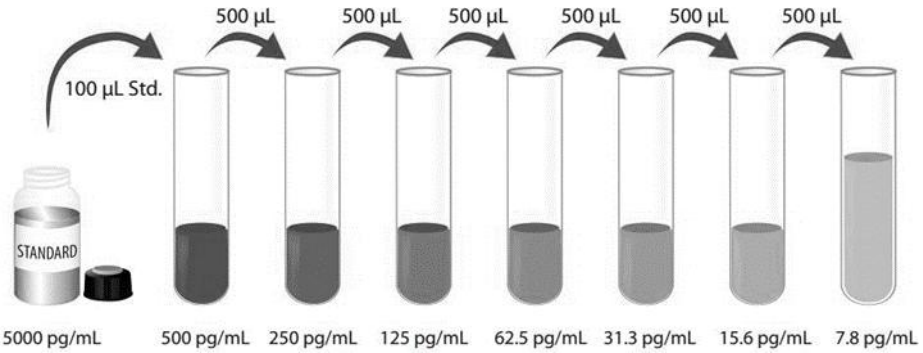
显色剂：按当次试验所需要用量将显色剂 A 和显色剂B 等体积混合，避光；在使用前15 分钟内准备，仅供当日使用；每孔需 200 µL。

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

标准品：依照标准品标签上注明的重溶体积重溶冻干标准品，得到浓度为 5000 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。

使用聚丙烯试管作为标准品稀释管。在 1000 pg/mL 的稀释管中加入 900 µL 稀释剂（1×），其余每个稀释管中加入 500 µL 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。没有稀释的标准品母液可用作标准曲线最高点（500 pg/mL），稀释剂（1×）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 200 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 1 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(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

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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