PRODUCT INFORMATION & MANUAL

Human/Monkey IL-10 Valukine™ ELISA

Catalog Number: VAL112

For the quantitative determination of natural and recombinant human/monkey 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

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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. An 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 antibody specific for IL-10 is added to the wells. Following a wash to remove any unbound antibody-enzyme reagent, TMB substrate solution (Chromogenic agent) 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 human/monkey 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 (1×) 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.

	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	7.6	6.6	5.6

B. RECOVERY

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

The recovery of IL-10 spiked to different levels throughout the range of the assay in human serum was evaluated. The recovery ranged from 77 to 85% with an average of 80%.

The recovery of IL-10 spiked to different levels throughout the range of the assay in monkey serum was evaluated. The recovery ranged from 73 to 105% with an average of 95%.

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 *Sf* 21-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	Human		Monkey	
	Average% of Expected	Range (%)	Average% of Expected	Range (%)
1:2	98	92 - 108	98	94 - 112
1:4	96	86 - 109	106	100 - 123
1:8	94	84 - 104	117	112 - 129
1:16	90	82 - 98	96	78 - 125

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 IL-10.

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

Monkey peripheral blood mononuclear cells (1×10^6 cells/mL) were cultured in RPMI 1640 supplemented with 10% fetal calf serum, 50 µM β-mercaptoethanol, 2 mM L-glutamine, 100 U/mL penicillin, and 100 µg/mL streptomycin sulfate and stimulated for 5 days with 10 µg/mL PHA. An aliquot of the cell culture supernate was removed, assayed for levels of IL-10, and measured 23.7 pg/mL.

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

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

G. SPECIFICITY

This assay recognizes both natural and recombinant IL-10. The following factors were prepared at 50 ng/mL in Calibrator Diluent (1×) and assayed for cross-reactivity. Preparations of the following factors at 50 ng/mL in a mid-range rhIL-10 control were

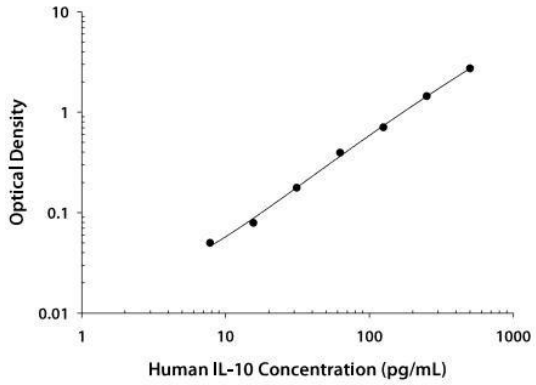
assayed for interference. No significant cross-reactivity or interference was observed.

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 an antibody against IL-10	1 plate
IL-10 Conjugate	A solution of antibody against IL-10 conjugated to horseradish peroxidase	1 vial
IL-10 Standard	Recombinant IL-10 in a buffered protein base; lyophilized. Refer to the vial label for reconstitution volume.	1 vial
Calibrator Diluent (5×)/RD5C	A 5× concentrated buffered protein base used to dilute standard and samples.	1 vial
Wash Buffer Concentrate (25×)	A 25× concentrated solution of buffered surfactant	1 vial
TMB Substrate	TMB ELISA Substrate Solution/TMB Substrate Solution	2 vials
Stop Solution	2 N sulfuric acid	1 vial
Plate Sealers	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="6">Opened/ Reconstituted Reagents</td><td>Wash Buffer (1×)</td><td rowspan="4">May be stored for up to 1 month at 2-8°C.*</td></tr>
<tr><td>Stop Solution</td></tr>
<tr><td>Conjugate</td></tr>
<tr><td>TMB Substrate</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>Calibrator Diluent (5×)/RD5C</td><td>May be stored for up to 1 month at 2-8 °C.*
Use and discard diluted Calibrator Diluent (1×). Prepare fresh for each assay.</td></tr>
<tr><td>Microplate Wells</td><td>Return unused wells to the foil pouch containing the desiccant pack, reseal along entire edge of zip-seal. May be stored for up to 1 month at 2-8°C.*</td></tr>
</table>

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

C. OTHER SUPPLIES REQUIRED

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

D. PRECAUTION

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

VI. PREPARATION

A. SAMPLE COLLECTION AND STORAGE

Cell Culture Supernates - Remove particulates by centrifugation and assay immediately or aliquot and store samples at ≤-20°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. Samples may require dilution with Calibrator Diluent (1×).

B. SAMPLE PREPARATION

Human serum samples recommend a 2-fold dilution. A suggested 2-fold dilution is 100 µL of sample + 100 µL of Calibrator Diluent (1×). Optimal dilutions should be determined by the end user.

Monkey serum samples recommend a 2-fold dilution. A suggested 2-fold dilution is 100 µL of sample + 100 µL of Calibrator Diluent (1×). Optimal dilutions should be determined by the end user.

C. REAGENT PREPARATION

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

Wash Buffer (1×) - 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 (1×).

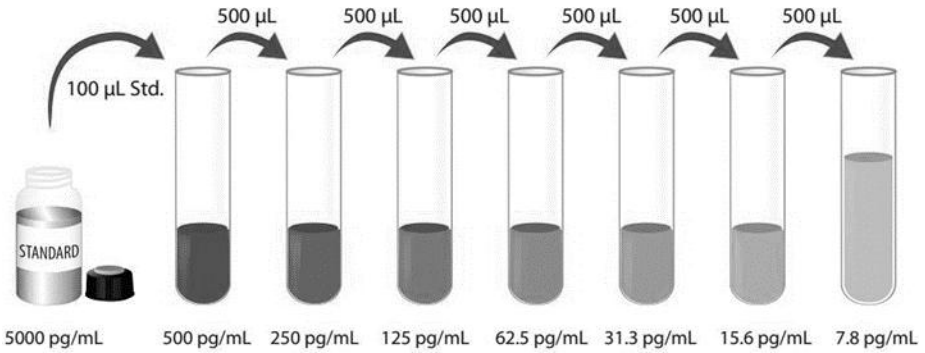
Calibrator Diluent (1×) - Use deionized or distilled water to prepare Calibrator Diluent (1×).

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.

Pipette 900 µL of Calibrator Diluent (1×) into 500 pg/mL tube. Pipette 500 µL of the appropriate Calibrator Diluent (1×) into each remaining 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 (1×) serves as the zero standard (0 pg/mL).



D. TECHNICAL HINTS

- When mixing or reconstituting protein solutions, always avoid foaming.
- To avoid cross-contamination, change pipette tips between additions of each standard level, between sample additions, and between reagent additions. Also, use separate reservoirs for each reagent.
- It is recommended that the samples be pipetted within 15 minutes.
- To ensure accurate results, proper adhesion of plate sealers during incubation steps is necessary.
- TMB Substrate should remain colorless until added to the plate. Keep TMB Substrate protected from light. TMB Substrate should change from colorless to gradations of blue.
- Stop Solution should be added to the plate in the same order as the TMB 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 TMB Substrate.

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 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 three times for a total of four washes. Wash by filling each well with Wash Buffer (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 TMB Substrate to each well. **Incubate for 30 minutes at room temperature. Protect from light.**
8. Add 50 µL of Stop Solution to each well. Gently tap the plate to ensure thorough mixing.
9. Determine the optical density of each well within 10 minutes, using a microplate reader set to 450 nm. If wavelength correction is available, set to 540 nm or 570 nm. If wavelength correction is not available, subtract readings at 540 nm or 570 nm from the readings at 450 nm. This subtraction will correct for optical imperfections in the plate. Readings made directly at 450 nm without correction may be higher and less accurate.
10. **CALCULATION OF RESULTS**

    Average the duplicate readings for each standard 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-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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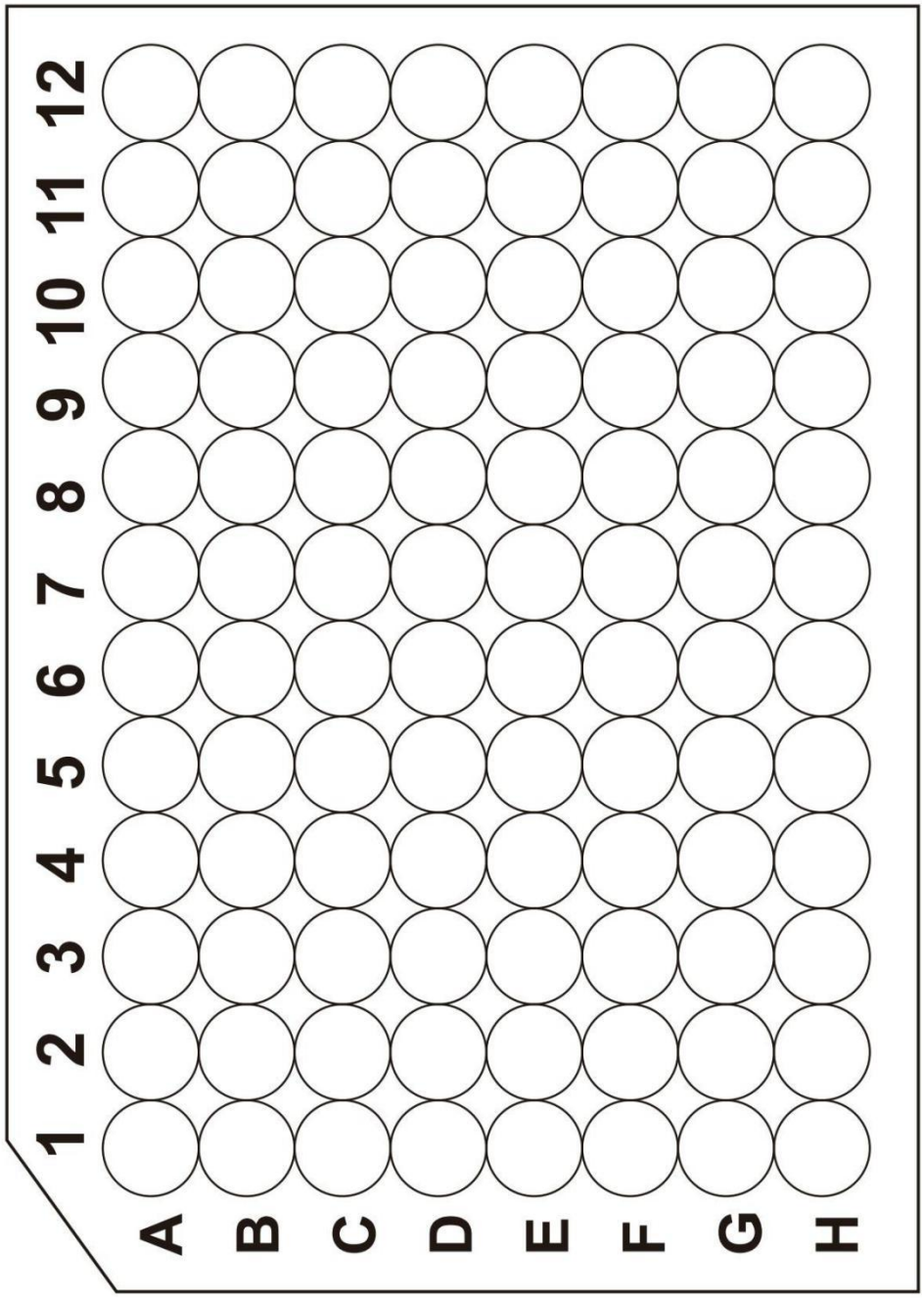
PLATE LAYOUT

Use this plate layout to record standards and samples assayed.



产品信息及操作手册

人/猴 IL-10 Valukine™ ELISA 试剂盒

目录号：VAL112

适用于定量检测天然和重组人/猴白介素 10（IL-10）的浓度

科研专用，不可用于临床诊断

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版本号 202403.5

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36 kDa（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110 kDa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75 kDa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IL-10抗体包被于微孔板上，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IL-10抗体，与结合在微孔板上的IL-10结合而形成免疫复合物，游离的成分被洗去；加入TMB底物溶液（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/猴血清样本；
- 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液（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、试剂盒的效期等。

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IL-10，测定其回收率。回收率范围在77-85%，平均回收率在80%。

在猴血清样本中掺入不同水平的IL-10，测定其回收率。回收率范围在73-105%，平均回收率在95%。

C. 灵敏度

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IL-10，然后用标准品稀释液（1×）将样本稀释到检测范围内，测定其线性。

稀释倍数	人		猴	
	平均值/期待值（%）	范围（%）	平均值/期待值（%）	范围（%）
1:2	98	92 - 108	98	94 - 112
1:4	96	86 - 109	106	100 - 123
1:8	94	84 - 104	117	112 - 129
1:16	90	82 - 98	96	78 - 125

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RPMI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

猴的外周血单核细胞（1×10^6细胞/mL）培养于含有10%胎牛血清的RPMI 1640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，另加10 μg/mL PHA刺激细胞，在5天时，取刺激后的细胞上清液测定IL-10含量，检测值为23.7 pg/mL。

人血清样本 - 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。

猴血清样本 - 使用本试剂盒检测了4份猴血清样本中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IL-10蛋白。将以下因子用稀释液（1×）配制成50 ng/mL的浓度来检测与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

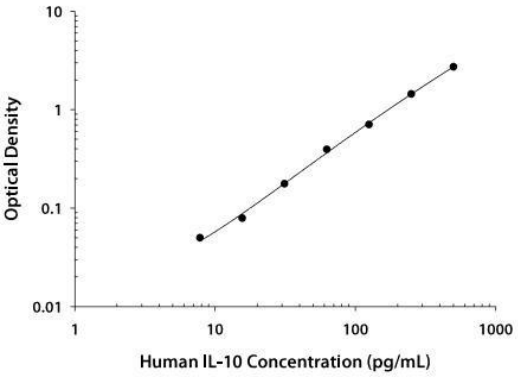
对照品中，来检测对IL-10的干扰。没有观察到明显的交叉反应或干扰。

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. 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。



(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IL-10的抗体的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），参考瓶标签进行重溶	1瓶
Calibrator Diluent (5×)/RD5C	浓缩的标准品稀释液（5×）用于稀释标准品和样本	1瓶
Wash Buffer Concentrate（25×）	浓缩洗涤缓冲液（25×）	1瓶
TMB Substrate	TMB ELISA底物溶液/TMB底物溶液	2瓶
Stop Solution	终止液	1瓶
Plate Sealers	封板膜	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="6">已打开，稀释或重溶的试剂</td><td>洗涤液（1×）</td><td rowspan="4">2-8℃储存，最多30天*</td></tr>
<tr><td>终止液</td></tr>
<tr><td>酶标检测抗体</td></tr>
<tr><td>TMB底物溶液</td></tr>
<tr><td>标准品</td><td>分装，<-20℃手动除霜储存最多30天*；避免反复冻融。</td></tr>
<tr><td>标准品稀释液(5×) /RD5C</td><td>2-8℃储存，最多30天*
请每次使用新鲜配制的1×标准品稀释液，多余的丢弃</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
- 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，使用时请做好眼睛、手、面部及衣服的防护。

VI. 实验前准备

A. 样品收集及储存

细胞培养上清液：颗粒物应离心去除；立刻检测样本。样本收集后若不及时检测，需按一次使用量分装，冻存于≤-20℃冰箱内，避免反复冻融。样本可能需要用标准品稀释液（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 × 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。样本可能需要用标准品稀释液（1×）稀释。

B. 样本准备工作

人血清样本建议用标准品稀释液（1×）2倍稀释后进行检测，即100 µL血清+100 µL标准品稀释液（1×）。最佳稀释度应由最终用户确定。

猴血清样本建议用标准品稀释液（1×）2倍稀释后进行检测，即100 µL血清+100 µL标准品稀释液（1×）。最佳稀释度应由最终用户确定。

C. 检测前准备工作

使用前请将所有试剂放置于室温

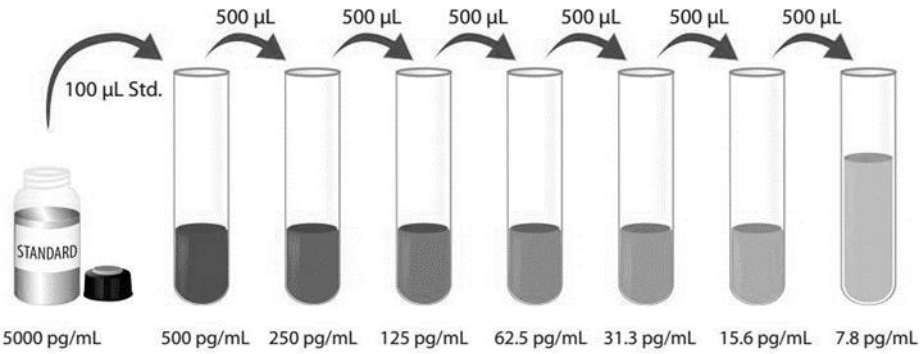
洗涤液（1×）：从冰箱中取出的浓缩洗涤液可能有结晶，属于正常现象；放置室温，轻摇混匀，待结晶完全溶解后再配制洗涤液。可将20 mL浓缩洗涤液用蒸馏水或去离子水稀释配制成500 mL工作浓度的洗涤液（1×）。

标准品稀释液（1×）：使用蒸馏水或去离子水稀释配制成标准品稀释液（1×）。

IL-10标准品：冻干标准品的重溶体积请参考瓶身标签，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。

在500 pg/mL的稀释管中加入900 µL标准品稀释液（1×），其余每个稀释管中加入500 µL标准品稀释液（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。 500 pg/mL管作标准曲线最高点，标准品稀释液（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，配制不同浓度标准品、上样、加不同试剂都需要更换枪头。另外不同试剂请分别使用不同的移液槽；
- 建议15分钟内完成一块板的上样；
- 每次孵育时，正确使用封板膜可保证结果的准确性；
- TMB底物溶液在上板前应为无色，请避光保存；加入微孔板后，TMB底物将由无色变成不同深度的蓝色；
- 终止液上板顺序应同TMB底物溶液上板顺序一致；加入终止液后，孔内颜色由蓝变黄；若孔内有绿色，则表明孔内液体未混匀，请充分混合。

VII. 操作步骤

使用前请将所有试剂和样本放置于室温，建议所有的实验样本和标准品做复孔检测

1. 按照上一节的说明，准备好所有需要的试剂和标准品；
2. 从已平衡至室温的密封袋中取出微孔板，未用的板条请放回铝箔袋内，重新封口；
3. 分别将不同浓度标准品或实验样本加入相应孔中，每孔200 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3次，共洗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。用封板膜封住反应孔，**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 TMB底物溶液，**室温孵育30分钟。注意避光；**
8. 在每个微孔内加入50 µL终止液，请轻拍微孔板，使溶液混合均匀；
9. 加入终止液后10分钟内，使用酶标仪测量450 nm的吸光度值，设定540 nm或570 nm作为校正波长。如果波长校正不可用，以450 nm的读数减去540 nm或570 nm的读数。这种减法将校正酶标板上的光学缺陷。没有校正而直接在450 nm处进行的读数可能会更高且更不准确；
10. **计算结果：**将每个标准品和样品的复孔吸光值取平均值，然后减去零标准品平均OD值（O.D.），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。另一替代方法是，通过绘制y轴上每个标准品的平均吸光值与x轴上的浓度来构建标准曲线，并通过图上的点绘制最佳拟合曲线。数据可以通过绘制IL-10浓度的对数与O.D.的对数来线性化，并且最佳拟合线可以通过回归分析来确定。该程序将产生足够但不太精确的数据拟合。

    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。

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