# PRODUCT INFORMATION & MANUAL

**Mouse IL-10 Valukine™ ELISA**

**Catalog Number: VAL605**

For the quantitative determination of natural and recombinant mouse 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 202409.4

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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 mouse IL-10 shares 85% amino acid sequence identity with rat IL-10 and 70-77% with bovine, canine, equine, feline, human, ovine, and porcine 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-10Rβ also associate with IL-20 Rα, IL-22 R α1, 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 (30-32).

# II. OVERVIEW

## A. PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. An antibody specific for mouse IL-10 has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any mouse IL-10 present is bound by the immobilized antibody. After washing away any unbound substances, an enzyme-linked antibody specific for mouse IL-10 is added to the wells. Following a wash to remove any unbound antibody-enzyme reagent, TMB substrate (Chromogenic agent) is added to the wells and color develops in proportion to the amount of mouse 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 mouse 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)

Two 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.

| | **Intra-assay Precision** | | **Inter-assay Precision** | | |
|---|---|---|---|---|---|
| Sample | 1 | 2 | 1 | 2 | 3 |
| Mean (pg/mL) | 76.3 | 123 | 815 | 71.4 | 115 |
| Standard Deviation | 2.56 | 2.85 | 17.0 | 5.39 | 7.64 |
| CV% | 3.4 | 2.3 | 2.1 | 7.5 | 6.6 |

## B. RECOVERY

The recovery of mouse IL-10 spiked to different levels throughout the range of the assay in cell culture media was evaluated. The recovery ranged from 95 to 103% with an average of 99%.

The recovery of mouse IL-10 spiked to different levels throughout the range of the assay in mouse serum was evaluated. The recovery ranged from 75.8 to 84.0% with an average of 80.3%.

## C. SENSITIVITY

The minimum detectable dose (MDD) of mouse IL-10 is typically less than 1.97 pg/mL.

The MDD was determined by adding two standard deviations to the mean optical density value of twenty zero standard replicates and calculating the corresponding concentration.

## D. CALIBRATION

This immunoassay is calibrated against highly purified *E. coli*-expressed recombinant mouse 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 mouse IL-10 and Calibrator Diluent (1×) to produce samples with values within the dynamic range of the assay.

| Dilution | Average % of Expected | Range (%) |
|---|---|---|
| 1:2 | 93 | 90-94 |
| 1:4 | 90 | 85-94 |
| 1:8 | 89 | 84-96 |
| 1:16 | 87 | 84-92 |

## F. SAMPLE VALUES

**Cell Culture Supernates -** Mouse spleens were removed, rinsed in 1× PBS, and kept on ice in PBS. Organs were then homogenized using a tissue homogenizer. Cells were seeded into media containing RPMI1640 supplemented with 10% fetal bovine serum, 2 mM L-glutamine, 100 U/mL penicillin and 100 ug/mL streptomycin sulfate. Cells were cultured unstimulated or stimulated with 1 µg/mL LPS for 3 days. Aliquots of the cell culture supernates were removed and assayed for levels of natural mouse IL-10.

| | Unstimulated (pg/mL) | Stimulated (pg/mL) |
|---|---|---|
| Spleen | Non-detectable | 77.1 |

Spleens from individual mice were removed and rinsed in PBS and kept on ice. The tissue was homogenized using a tissue homogenizer and seeded into media containing RPMI1640 supplemented with 10% fetal bovine serum, 50 µM β-mercaptoethnal, 2mM L-glutamine, 100U/mL penicillin and 100 µg/mL streptomycin sulfate. Cells were stimulated with 100 ng/mL recombinant mouse IFN-γ and 1 µg/mL LPS for 4 days. An aliquot of the cell culture supernate was removed, assayed for detectable levels of natural mouse IL-10, and measured 515 pg/mL.

**Mouse serum -** Four mouse serum samples were evaluated for the presence of mouse IL-10 in this assay. All samples measured below the lowest standard, 15.6 pg/mL.

## G. SPECIFICITY

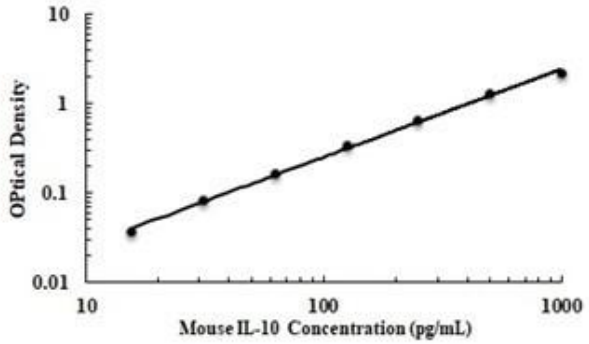
This assay recognizes both natural and recombinant mouse IL-10. The following factors were prepared at 50 ng/mL and assayed for cross-reactivity. Preparations of the following factors at 50 ng/mL in a mid-range recombinant mouse IL-10 control were assayed for interference. No significant cross-reactivity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

| **Recombinant mouse** | | **Other Recombinants** |
|---|---|---|
| IFN-α/β R2 | IL-19 | canine IL-10 |
| IFN-γ R1 | IL-20 | equine IL-10 |
| IFN-γ R2 | IL-22 | feline IL-10 |
| IL-10 R | IL-22 R | human IL-10 |
| IL-10 Rα | IL-24 | porcine IL-10 |
| IL-10 Rβ | | viral IL-10 |

# 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 | OD | Average | Corrected |
|---|---|---|---|
| 0 | 0.050<br>0.049 | 0.050 | - |
| 15.6 | 0.091<br>0.083 | 0.087 | 0.037 |
| 31.3 | 0.134<br>0.127 | 0.131 | 0.081 |
| 62.5 | 0.219<br>0.209 | 0.214 | 0.164 |
| 125 | 0.386<br>0.386 | 0.386 | 0.336 |
| 250 | 0.715<br>0.697 | 0.706 | 0.656 |
| 500 | 1.318<br>1.312 | 1.315 | 1.265 |
| 1000 | 2.266<br>2.255 | 2.261 | 2.211 |

# V. KIT COMPONENTS AND STORAGE

## A. MATERIALS PROVIDED

| Parts | Description | Size |
|---|---|---|
| Mouse IL-10 Microplate | 96 well polystyrene microplate (12 strips of 8 wells) coated with antibody against mouse IL-10 | 1 plate |
| Mouse IL-10 Conjugate | Solution of an antibody against mouse IL-10 conjugated to horseradish peroxidase | 1 vial |
| Mouse IL-10 Standard | Recombinant mouse IL-10 in a buffered protein base; lyophilized. Refer to the vial label for reconstitution volume. | 2 vials |
| Calibrator Diluent (5×)/RD5P | 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 | 1 vial |
| Stop Solution | Diluted hydrochloric acid solution | 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/<br>Reconstituted<br>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>Use a new standard for each assay. Discard after use.<br>Standards may be stored for up to 1 month at -20 °C.*</td></tr>
<tr><td>Calibrator Diluent (5×)/RD5P</td><td>May be stored for up to 1 month at 2-8 °C.*<br>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 oil ouch 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.
- Horizontal orbital microplate shaker (0.12” orbit) capable of maintaining a speed of 500(±) 50 rpm.
- Test tubes for dilution of standards and samples.

## D. PRECAUTION

- Some components in this kit contain a preservative which may cause an allergic skin reaction. Avoid breathing mist.
- 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 × 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

Mouse serum samples recommend a 2-fold dilution. A suggested 2-fold dilution is 100 µL of mouse serum 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.

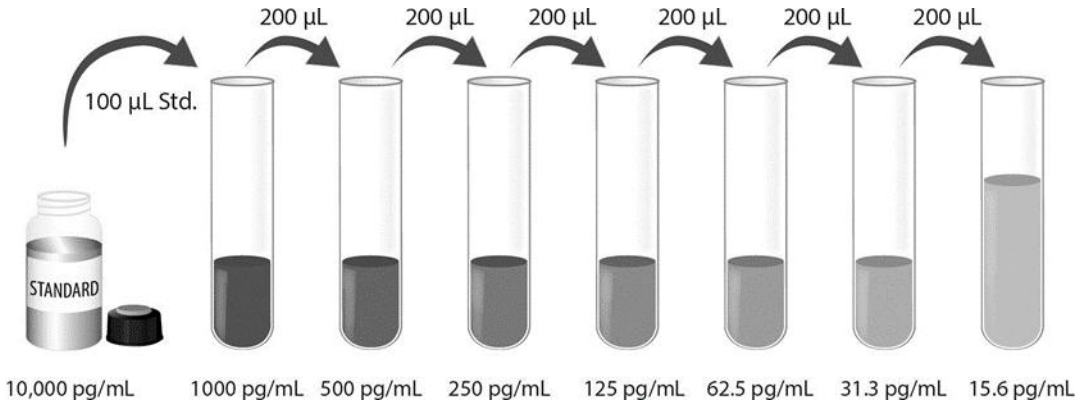
**Wash Buffer (1×)** - If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. Dilute 20 mL of Wash Buffer Concentrate (25×) into deionized or distilled water to prepare 500 mL of Wash Buffer (1×).

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

**Mouse IL-10 Standard - Refer to the vial label for reconstitution volume*.** This reconstitution produces a stock solution of 10,000 pg/mL. Allow the standard to sit for a minimum of 5 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 the 1000 pg/mL tube.** Pipette 200 µL of Calibrator Diluent (1×) into the remaining tubes. Use the stock solution to produce a dilution series (below). Mix each tube thoroughly before the next transfer. The standard 1000 pg/mL serves as the high standard. The 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. 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. A. CELL CULTURE SUPERNATE SAMPLES

   *Add 50 µL of Calibrator Diluent (1×) to each well. And then add 50 µL of Standard and cell culture supernate sample using Calibrator Diluent (1×) per well.*

   B. MOUSE SERUM SAMPLES

   *Add 100 µL of Standard and mouse serum sample using Calibrator Diluent (1×) per well.*
4. Cover with the adhesive strip provided. **Incubate for 2 hours at room temperature on a horizontal orbital microplate shaker (0.12" orbit) set at 500±50 rpm.** A plate layout is provided for a record of standards and samples assayed.
5. 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.
6. Add 100 µL of mouse IL-10 conjugate to each well. Cover with a new adhesive strip. **Incubate for 2 hours at room temperature on a horizontal orbital microplate shaker (0.12" orbit) set at 500±50 rpm.**
7. Repeat the aspiration/wash as in step 5.
8. Add 100 µL of TMB Substrate to each well. **Incubate for 30 minutes at room temperature. Protect from light**.
9. Add 100 µL of Stop Solution to each well. Gently tap the plate to ensure thorough mixing.

10. 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.

11. **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 mouse 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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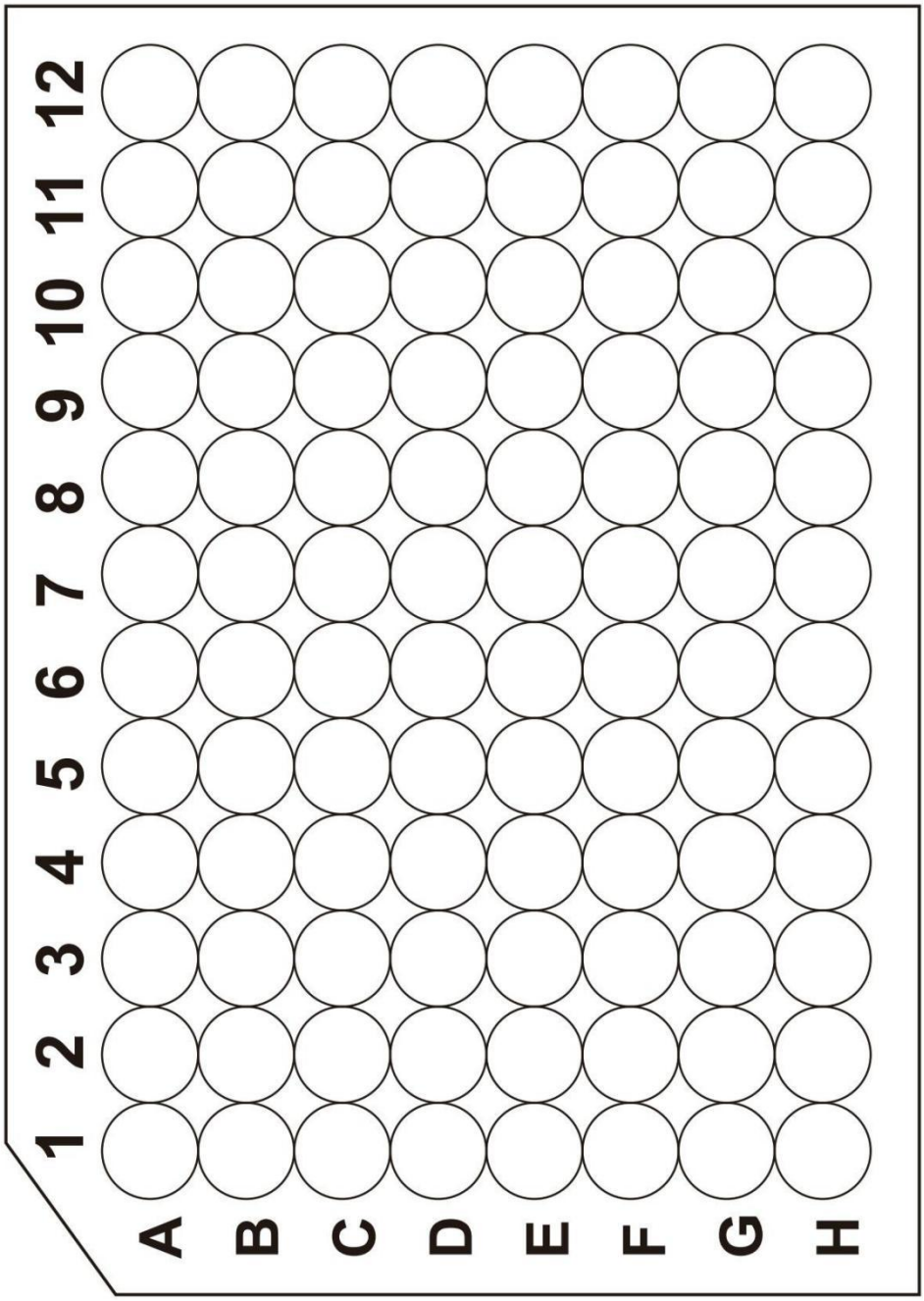
## PLATE LAYOUT

Use this plate layout to record standards and samples assayed.



# 产品信息及操作手册

## 小鼠 IL-10 Valukine™ ELISA 试剂盒

## 目录号：VAL605

适用于定量检测天然和重组小鼠 IL-10 的浓度

科研专用，不可用于临床诊断

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版本号 202409.4

# 目录

# I. 背景

小鼠白细胞介素-10 (IL-10)又称为细胞因子合成抑制因子(CSIF)。它是IL-10 α-螺旋细胞因子家族成员之一，该分子家族中还包括IL-19、IL-12、IL-22、IL-24以及IL-26/AK155 (1-3)。许多激活的造血干细胞、肝脏星状细胞、角质化细胞和胎盘细胞滋养层均分泌IL-10。人IL-10在小鼠细胞中具有活性，但小鼠IL-10却不作用于人类细胞（4, 5）。成熟小鼠IL-10与大鼠IL-10的氨基酸同源性为85%，与牛、犬、马、猫、人、羊、猪的氨基酸同源性为70~77%。成熟小鼠IL-10是一个分子量为36 kDa的非共价结合的同源二聚体，含有两个链内二硫键（4, 6, 7）。

IL-10通过由II型细胞因子受体IL-10 Rα和IL-10β组成的异聚体受体复合物发挥其生物活性。IL-10 Rα是一个110 kDa的跨膜糖蛋白，在淋巴细胞、天然杀伤细胞、巨噬细胞、单核细胞、星形胶质细胞、肠上皮细胞、细胞滋养层以及活化的肝星状细胞中表达（8-13），而75 kDa的IL-10Rβ却广泛表达于生物体内（14, 15）。IL-10二聚体先结合两个IL-10 Rα链，进一步结合两个IL-10 Rβ链(14, 15)。IL-10 Rβ不直接与IL-10结合，但参与信号转导。IL-10 Rβ分别与IL-20 Rα、IL-22 Rα1和IL-28 Rα形成IL-22、IL-26、IL-28和IL-29的受体复合物（16-18）。

IL-10参与抑制和激活作用的免疫调节。IL-10作为抗炎症的细胞因子是通过抑制Th1 细胞和Th17细胞的激活和扩张（19-21），同时促进M2巨噬细胞的形成（21）。免疫抑制调节T细胞和B细胞的表达对Treg细胞增殖有重要作用(19)。在肿瘤微环境中，IL-10抑制调节T细胞和髓系来源抑制细胞的增殖（22, 23）。IL-10诱导瘤内物质的累积并激活$CD8^+$ T细胞（24, 25）。IL-10在限制关节炎症中的组织损伤，促进损伤后肌肉再生长过程中发挥重要保护作用，但也促成持久性病毒感染（19, 21, 26）。在修格兰氏症候群（唾液）和原发性中枢神经系统淋巴瘤中IL-10的含量有所升高（30-32）。

# II. 概述

## A. 检测原理

本实验采用双抗体夹心ELISA法。抗小鼠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小鼠IL-10抗体，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小鼠血清样本；
- 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）

已知浓度的两个样本，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。

| | 板内精确度 | | 板间精确度 | | |
|---|---|---|---|---|---|
| 样本 | 1 | 2 | 1 | 2 | 3 |
| 平均值 (pg/mL) | 76.3 | 123 | 815 | 71.4 | 115 |
| 标准差 | 2.56 | 2.85 | 17.0 | 5.39 | 7.64 |
| CV% | 3.4 | 2.3 | 2.1 | 7.5 | 6.6 |

### 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小鼠IL-10，测定其回收率。回收率范围在95-103%， 平均回收率在99%。

在小鼠血清样本中掺入检测范围内不同水平的小鼠IL-10，测定其回收率。回收率范围在75.8-84.0%，平均回收率在80.3%。

### C. 灵敏度

小鼠IL-10的最低可测剂量（MDD）一般小于1.97pg/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R&D Systems生产的大肠杆菌表达的高纯度重组小鼠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小鼠 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 | 平均值/期待值（%） | 范围 (%) |
|---|---|---|
| 1:2 | 93 | 90 - 94 |
| 1:4 | 90 | 85 - 94 |
| 1:8 | 89 | 84 - 96 |
| 1:16 | 87 | 84 - 92 |

## F. 样本预值

**细胞培养上清液 -** 以下的小鼠脾脏组织匀浆，其原代细胞培养于含有10%胎牛血清的100 mL的RPMI1640培养基中，同时还含有2 mM L-谷氨酰氨、100 U/mL青霉素、100 µg/mL链霉素。细胞分别未经和经有1 µg/mL LPS刺激3天。取细胞培养上清测定小鼠IL-10含量，结果见下表。

| 原代细胞种类 | 未刺激 (pg/mL) | 刺激组 (pg/mL) |
|---|---|---|
| 脾 | - | 77.1 |

小鼠脾脏组织经PBS冲洗后置于冰上。组织匀浆后，其原代细胞培植于含有10%胎牛血清、50 µM β-硫基乙醇、2 mM L-谷氨酰胺、100 U/mL青霉素和100 µg/mL链霉素的RPMI 1640培养基中。细胞经由100 ng/mL的重组小鼠IFN-γ和1 µg/mL LPS刺激4天。取细胞培养上清测定小鼠IL-10含量，结果为515 pg/mL。

**小鼠血清样本 -** 使用本试剂盒检测了4份小鼠血清样本中小鼠IL-10的水平。所有样本的检测值均低于最低标准品，15.6 pg/mL。

## G. 特异性

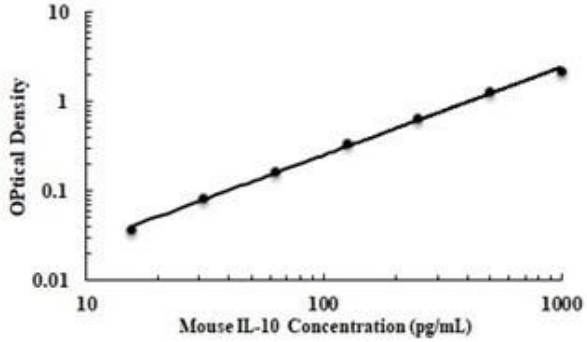
此ELISA法可检测天然及重组小鼠IL-10蛋白。将以下因子配制成50 ng/mL的浓度来检测与小鼠IL-10的交叉反应。将50 ng/mL的干扰因子掺入中间范围的重组小鼠IL-10对照品中，检测对小鼠IL-10的干扰。没有观察到明显的交叉反应或干扰。

| **Recombinant mouse** | | **Other Recombinants** |
|---|---|---|
| IFN-α/β R2 | IL-19 | canine IL-10 |
| IFN-γ R1 | IL-20 | equine IL-10 |
| IFN-γ R2 | IL-22 | feline IL-10 |
| IL-10 R | IL-22 R | human IL-10 |
| IL-10 Rα | IL-24 | porcine IL-10 |
| IL-10 Rβ | | viral IL-10 |

# 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 | OD | Average | Corrected |
|---|---|---|---|
| 0 | 0.050<br>0.049 | 0.050 | - |
| 15.6 | 0.091<br>0.083 | 0.087 | 0.037 |
| 31.3 | 0.134<br>0.127 | 0.131 | 0.081 |
| 62.5 | 0.219<br>0.209 | 0.214 | 0.164 |
| 125 | 0.386<br>0.386 | 0.386 | 0.336 |
| 250 | 0.715<br>0.697 | 0.706 | 0.656 |
| 500 | 1.318<br>1.312 | 1.315 | 1.265 |
| 1000 | 2.266<br>2.255 | 2.261 | 2.211 |

# V. 试剂盒组成及储存

## A. 试剂盒组成

| 组成 | 描述 | 规格 |
|---|---|---|
| Mouse IL-10 Microplate | 包被抗小鼠IL-10抗体的96孔聚苯乙烯板，8孔×12条 | 1块板 |
| Mouse IL-10 Conjugate | 酶标检测小鼠IL-10抗体 | 1瓶 |
| Mouse IL-10 Standard | 小鼠IL-10标准品（冻干），参考瓶身标签进行重溶 | 2瓶 |
| Calibrator Diluent (5×)/RD5P | 浓缩标准品稀释液（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 | 1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>每次新鲜使用，即用即弃。<br>标准品-20℃储存，最多30天*</td></tr>
<tr><td>标准品稀释液（5×）/ RD5P</td><td>2-8℃储存，最多 30 天*<br>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；<br>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；
- 水平微孔板振荡器（轨道直径3 mm），转速500±50 rpm；
- 标准品或标本稀释管。

## D. 注意事项

- 试剂盒中的一些组分含有防腐剂，可能引起皮肤过敏反应，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

小鼠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小鼠血清+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。**

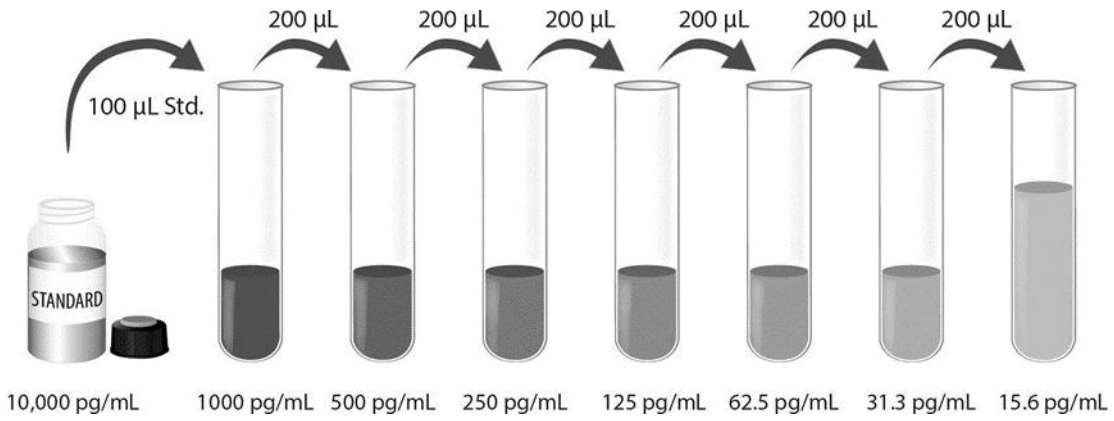
**洗涤液（1×）：**从冰箱中取出的浓缩洗涤液可能有结晶，属于正常现象；放置室温，轻摇混匀， 待结晶完全溶解后再配制洗涤液。可将20 mL浓缩洗涤液（25×）用蒸馏水或去离子水稀释配制成500 mL工作浓度的洗涤液（1×）。

**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×）。

**小鼠IL-10标准品：**重溶体积请参考瓶身标签，得到浓度为10,000 pg/mL标准品母液。轻轻震摇至少5分钟，其充分溶解。

*如有疑问，请咨询我们的技术支持。

**取900 µL标准品稀释液（1×）**至1000 pg/mL稀释管中，其他每个稀释管中加入200 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。标准品1000 pg/mL可用作标准曲线最高点，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，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. A. 细胞培养上清样本

   *在每个微孔中加入50 µL标准品稀释液（1×）；并分别在相应孔中加入每孔50 µL用标准品稀释液（1×）稀释的不同浓度标准品和细胞培养上清实验样本。*

   B. 小鼠血清样本

   *分别将用标准品稀释液（1×）稀释的不同浓度标准品和小鼠血清实验样本加入相应孔中，每孔100 µL。*

4. 用封板膜封住反应孔，**放置于水平微孔板振荡器（轨道直径3 mm）上，设置转速500±50 rpm，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；
5. 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；
6. 在每个微孔内加入100 µL酶标检测抗体。用封板膜封住反应孔，**放置于水平微孔板振荡器（轨道直径3 mm）上，设置转速500±50 rpm，室温孵育2小时**；
7. 重复第5步洗板操作；
8. 在每个微孔内加入100 µL TMB底物溶液，**室温孵育30分钟。注意避光**；
9. 在每个微孔内加入100 µL终止液，请轻拍微孔板，使溶液混合均匀；
10. 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；
11. **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。数据可以通过绘制小鼠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。

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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