PRODUCT INFORMATION & MANUAL

Mouse IL-10 Valukine™ ELISA

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

Version201912.2

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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 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. A monoclonal 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 polyclonal antibody specific for mouse 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 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 DIAGNOSTICPROCEDURES.**
- 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 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.

CELL CULTURE SUPERNATE / MOUSE SERUM

	Intra-assay Precision		**Inter-assay Precision**		
Sample	1	2	1	2	3
n	20	20	20	20	20
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 95-103% 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 75.8-84.0% 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, samples were spiked with high concentrations of mouse IL-10 and Calibrator Diluent (*for cell culture supernate samples*) or Calibrator Diluent-S (*for mouse serum samples*) 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 pg/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, 2 mM L-glutamine, 100 U/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.

Serum - Four mouse serum samples were evaluated for the presence of 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.

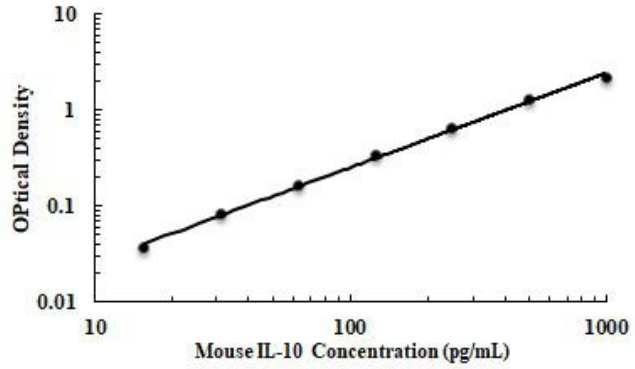
Recombinant mouse		**Other Recombinants**
IFN-α/β R2	IL-19	canine IL-10
IFN-γ R1	IL-20	equine IL-10
IFN-γ R2	IL-22	feline IL-10
IL-10 R	IL-22 R	human IL-10
IL-10 Rα	IL-24	porcine IL-10
IL-10 Rβ		viral IL-10

IV. EXPERIMENT

A. EXAMPLE STANDARD

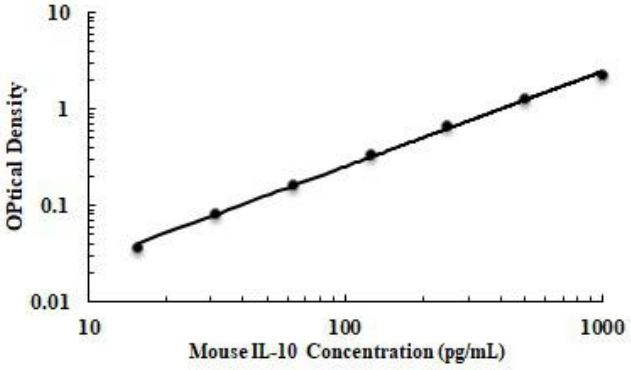
The standard curve is provided for demonstration only. A standard curve should be generated for each set of samples assayed.

CELL CULTURE SUPERNATE ASSAY



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

SERUM ASSAY



pg/ml	OD	Average	Corrected
0	0.102 0.100	0.101	-
15.6	0.144 0.139	0.142	0.041
31.3	0.178 0.171	0.175	0.074
62.5	0.271 0.282	0.277	0.176
125	0.448 0.436	0.442	0.341
250	0.797 0.778	0.788	0.687
500	1.379 1.329	1.354	1.253
1000	2.285 2.120	2.203	2.102

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 monoclonal antibody against mouse IL-10	1 plate
Mouse IL-10 Conjugate	solution of a polyclonal 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	2 vials
Calibrator Diluent（5×）	a 5× concentrated buffered protein base used to dilute standard and cell culture supernate samples	1 vial
Calibrator Diluent-S (2×)	a 2× concentrated buffered protein base used to dilute standard and mouse serum samples	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	diluted hydrochloric acid solution	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 Dilution (1×)</td></tr>
<tr><td>Calibrator Diluent-S (1×)</td></tr>
<tr><td>Conjugate</td></tr>
<tr><td>Unmixed color reagent A</td></tr>
<tr><td>Unmixed Color reagent B</td><td rowspan="2">Use a new standard and control for each assay. Discard after use.</td></tr>
<tr><td>Standard</td></tr>
<tr><td></td><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

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.

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

Mouse serum samples require a 2-fold dilution. A suggested 2-fold dilution is 100 µL of mouse serum sample + 100 µL of Calibrator Diluent-S (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 - Substrates A and 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.

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×).

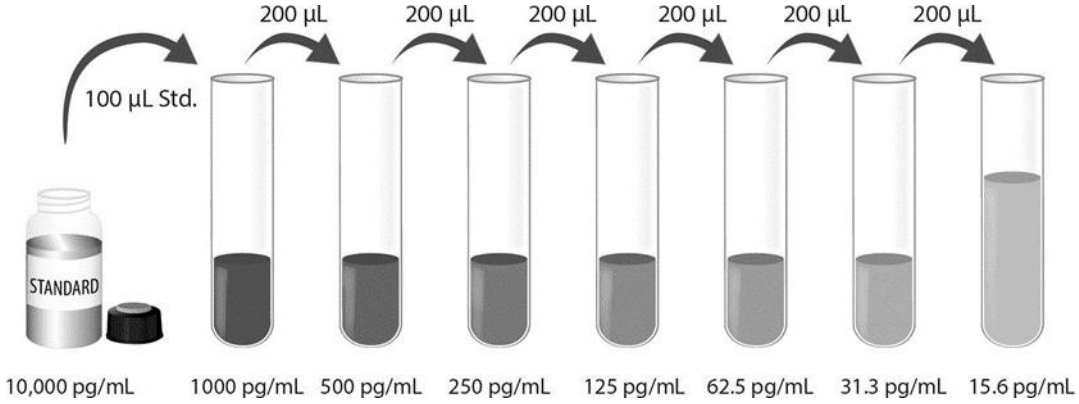
Calibrator Diluent-S (1×) - Use deionized or distilled water to prepare Calibrator Diluent-S (1×).

Mouse IL-10 Standard - Refer to the vial label for reconstitution volume* using Calibrator Diluent (1×) (*for cell culture supernate samples*) or Calibrator Diluent-S (1×) (*for mouse serum samples*). 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×) (*for cell culture supernate samples*) or Calibrator Diluent-S (1×) (*for mouse serum samples)* into the 1000 pg/mL tube. Pipette 200 µL of Calibrator Diluent (1×) (*for cell culture supernate samples*) or Calibrator Diluent-S (1×) (*for serum samples*) 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×) (*for cell culture supernate samples*) or Calibrator Diluent-S (1×) (*for serum samples*) 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. A. CELL CULTURE SUPERNATE SAMPLES

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

   B. MOUSE SERUM SAMPLES

   Add 100 µL of Standard, mouse serum sample, or control using Calibrator Diluent-S (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 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.
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 Substrate Solution to each well. Incubate for 30 minutes at room temperature. **Protect from light**.
9. Add 100 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.
10. 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.

11. **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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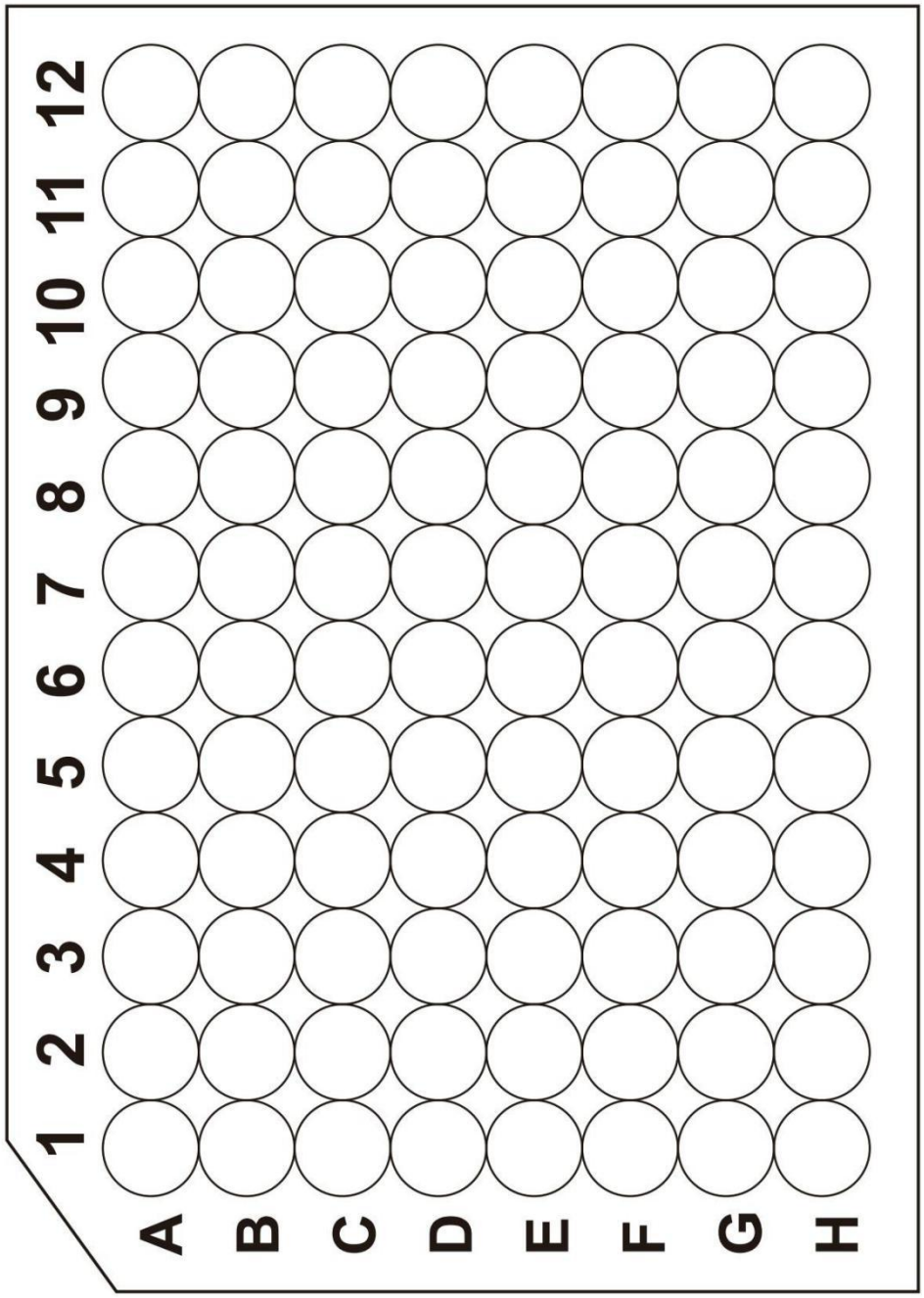
PLATE LAYOUT

Use this plate layout to record standards and samples assayed.



产品信息及操作手册

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 的含量

科研专用，不可用于临床诊断

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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。

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（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。

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 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小鼠 IL-10 多抗，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；加入底物溶液（显色剂），溶液颜色逐渐变成蓝色，加入终止液溶液变黄并且停止变化。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。

细胞培养上清/小鼠血清

	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%。

灵敏度

小鼠 IL-10 的最低可测剂量（MDD）一般小于 1.8 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。

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。

C. 线性

不同的细胞上清/小鼠血清中掺入高浓度的小鼠 IL-10，然后用稀释剂（1×）（*用于细胞上清样本*）或稀释剂-S（1×）（*用于小鼠血清样本*）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

D. 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 100mL 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。

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

E. 特异性

此 ELISA 法可检测天然及重组小鼠 IL-10 蛋白。将以下因子用稀释剂（1×）配置成 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。

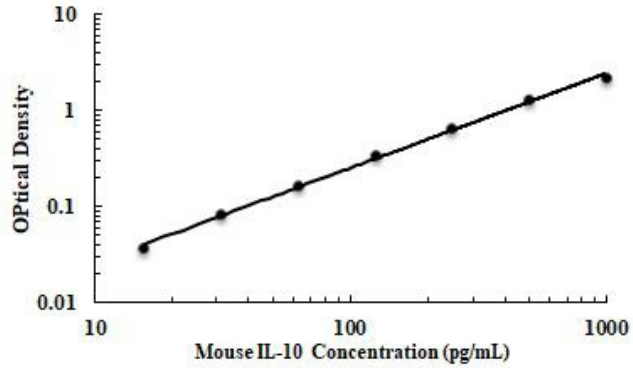
重组小鼠蛋白		其他重组蛋白
IFN-α/β R2	IL-19	canine IL-10
IFN-γ R1	IL-20	equine IL-10
IFN-γ R2	IL-22	feline IL-10
IL-10 R	IL-22 R	human IL-10
IL-10 Rα	IL-24	porcine IL-10
IL-10 Rβ		viral IL-10

IV. 实验标准

A. 标准曲线实例

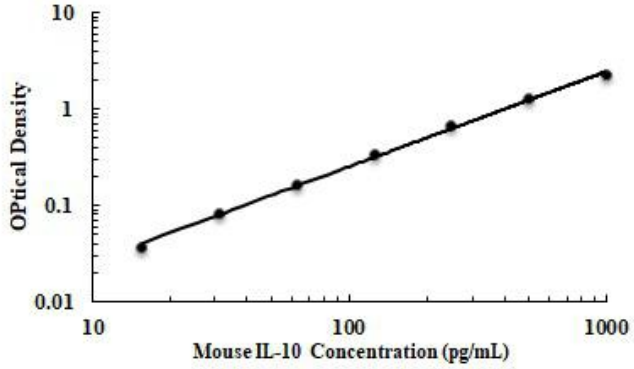
该标准曲线数据仅供参考，每次实验应绘制其对应的标准曲线。

CELL CULTURE SUPERNATE ASSAY



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

SERUM ASSAY



pg/ml	OD	Average	Corrected
0	0.102 0.100	0.101	-
15.6	0.144 0.139	0.142	0.041
31.3	0.178 0.171	0.175	0.074
62.5	0.271 0.282	0.277	0.176
125	0.448 0.436	0.442	0.341
250	0.797 0.778	0.788	0.687
500	1.379 1.329	1.354	1.253
1000	2.285 2.120	2.203	2.102

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	包被抗体的 96 孔聚苯乙烯板，8 孔×12 条	1 块板
Mouse IL-10 Conjugate	酶标检测IL-10 抗体	1 瓶
Mouse IL-10 Standard	标准品（冻干）	2 瓶
Calibrator Diluent（5×）	浓缩稀释剂（5×），用于稀释标准品和细胞上清样本	1 瓶
Calibrator Diluent-S（2×）	稀释剂-S（2×），用于稀释标准品和小鼠血清样本	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

* 本试剂盒包含足够的试剂以用于一块 96 孔微孔板的ELISA 实验。

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>稀释剂-S（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><td>每次新鲜使用，即用即弃。</td></tr>
<tr><td>标准品</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>
<tr><td>包被的微孔板条</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 3mm），转速 500 ± 50 rpm；
- 标准品或标本稀释管。

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

小鼠血清样本需要用稀释剂-S（1×）2 倍稀释后进行检测，即 100 µL 小鼠血清+ 100 µL 稀释剂-S（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 500 mL 工作浓度的洗涤液。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 100 µL。

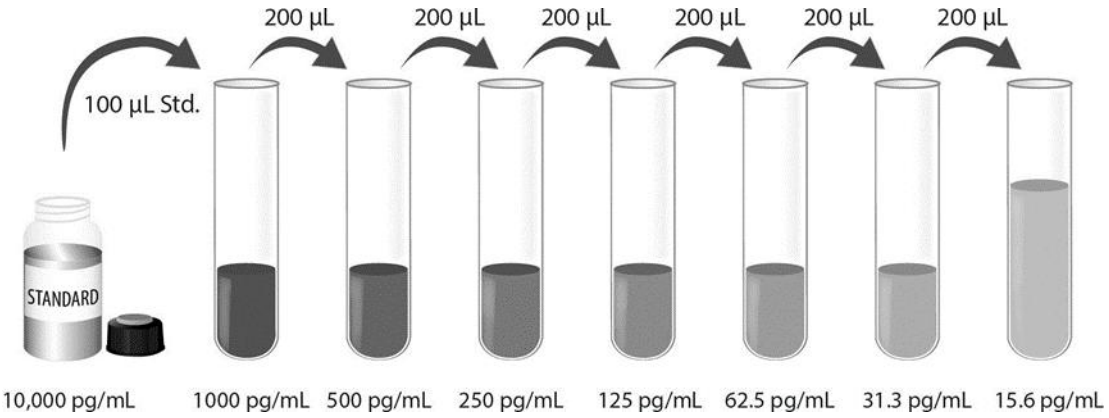
稀释剂（1×）：可将 20mL 浓缩稀释剂用 80mL 蒸馏水或去离子水稀释配置成 100 mL 工作浓度的稀释剂。

稀释剂-S（1×）：使用蒸馏水或去离子水稀释配置成试剂稀释剂-S（1×）。

标准品：重溶体积请参考瓶身标签，稀释剂（1×）（*用于细胞上清样本*）或稀释剂-S（1×）（*用于小鼠血清样本*），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 稀释剂（1×）（*用于细胞上清样本*）或稀释剂-S（1×）（*用于小鼠血清样本*）。至1000 pg/mL 稀释管中，其他每个稀释管中加入 200µL 稀释剂（1×）（*用于细胞上清样本*）或稀释剂-S（1×）（*用于小鼠血清样本*）。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，稀释剂（1×）（*用于细胞上清样本*）或稀释剂-S（1×）（*用于小鼠血清样本*）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. A. 细胞培养上清样本

   在每个微孔中加入 50 µL 稀释剂(1×)；并分别在相应孔中加入每孔 50 µL 用稀释剂(1×)稀释的不同浓度标准品，细胞培养上清实验样本或者质控品。

   B. 小鼠血清样本

   分别将用稀释剂S（1×）稀释的不同浓度标准品，小鼠血清实验样本或者质控品加入相应孔中，每孔 100 µL。
4. 用封板胶纸封住反应孔，放置于水平微孔板振荡器（轨道直径3mm）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；
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 100 µL 酶标检测抗体。用封板胶纸封住反应孔，放置于水平微孔板振荡器（轨道直径 3mm）上，设置转速 500 ± 50 rpm，室温孵育 2 小时；
6. 重复第 5 步洗板操作；
7. 在每个微孔内加入 100 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 100 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