PRODUCT INFORMATION & MANUAL

Mouse IL-6 Valukine™ ELISA

Catalog Number: VAL604G

For the quantitative determination of natural and recombinant
Mouse IL-6 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 202307.2

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I. BACKGROUND

Interleukin 6 (IL-6) is a pleiotropic a-helical 22-28 kDa phosphorylated and variably glycosylated cytokine that plays important roles in acute phase reactions, inflammation, hematopoiesis, bone metabolism, and cancer progression (1-5). Mature Mouse IL-6 is 187 amino acids (aa) in length and shares 42% and 85% as sequence identity with human and rat IL-6, respectively (6). Alternate splicing generates several isoforms with internal deletions some of which exhibit antagonistic properties (7-10). Cells known to express IL-6 include $CD8^+$ T cells, fibroblasts, synoviocytes, adipocytes, osteoblasts, megakaryocytes, endothelial cells (under the influence of endothelin), sympathetic neurons, cerebral cortex neurons, adrenal medulla chromaffin cells, retinal pigment cells, mast cells, keratinocytes, Langerhans cells, fetal and adult astrocytes, neutrophils, monocytes, eosinophils, colonic epithelial cells, B1 B cells and pancreatic islet beta cells (2,7,10-33). IL-6 production is generally correlated with cell activation and is normally kept in control by glucocorticoids, catecholamines and secondary sex steroids (2). Normal human circulating IL-6 is in the 1 pg/mL range, with slight elevations during the menstrual cycle, modest elevations in certain cancers, and large elevations after surgery (34-38).

IL-6 induces signaling through a cell surface heterodimeric receptor complex composed of a ligand binding subunit (IL-6R) and a signal transducing subunit (gp130). IL-6 binds to IL-6R, triggering IL-6R association with gp130 and gp130 dimerization (39). Gp130 is also a component of the receptors for CLC, CNTF, CT-1, IL-11, IL-27, LIF, and OSM (40). Soluble forms of IL-6R are generated by both alternate splicing and proteolytic cleavage (3). In a mechanism known as trans-signaling, complexes of soluble IL-6 and IL-6R elicit responses from gp130-expressing cells that lack cell surface IL-6R (1, 3). Trans-signaling enables a wider range of cell types to respond to IL-6, as the expression of gp130 is ubiquitous, while that of IL-6R is predominantly restricted to hepatocytes, monocytes, and resting lymphocytes (1-3). Soluble splice forms of gp130 block trans-signaling from IL-6/IL-6R but not from other cytokines that use gp130 as a co-receptor (3, 41).

IL-6, along with TNF-α and IL-1, drives the acute inflammatory response, is almost solely responsible for fever and the acute phase response in the liver, and is important

in the transition from acute inflammation to either acquired immunity or chronic inflammatory disease (1-4). It contributes to chronic inflammation in conditions such as obesity, insulin resistance, inflammatory bowel disease, inflammatory arthritis and sepsis when dysregulated, often involving IL-6 trans-signaling (1, 2). It also plays an important role in the differentiation of naive T cells to Th17 inflammatory cells in the presence of TGF-β. IL-6 modulates bone resorption and is a major effector of inflammatory joint destruction in rheumatoid arthritis through its promotion of Th17 T cell activity (1). It contributes to atherosclerotic plaque development and destabilization (2). However, IL-6 can also have anti-inflammatory effects, such as in skeletal muscle where it is secreted in response to exercise (2). It promotes hematopoiesis by being a growth factor for hematopoietic stem cells, induces B cell maturation to plasma cells and perpetuates multiple myeloma (1, 42). IL-6 also promotes, but probably does not initiate, other types of inflammation-associated carcinogenesis, such as colitis-associated cancer (1).

II. OVERVIEW

A. PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for mouse IL-6 has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any mouse IL-6 present is bound by the immobilized antibody. After washing away any unbound substances, a biotinylated detection antibody specific for mouse IL-6 is pipetted into the wells. After washing away any unbound substances, streptavidin-HRP is pipetted into the wells. Following a wash to remove any unbound reagent, TMB substrate is added to the wells and color develops in proportion to the amount of mouse IL-6 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, mouse serum and plasma.
- 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 twenty separate assays to assess inter-assay precision.

	Intra-assay Precision			Inter-assay Precision		
Sample	1	2	3	1	2	3
Mean (pg/mL)	245.8	62.4	15.2	247.7	61.9	15.1
Standard Deviation	10.4	2.8	0.7	9.7	2.6	0.6
CV%	4.2	4.5	4.5	3.9	4.2	4.0

B. RECOVERY

The recovery of mouse IL-6 spiked to levels throughout the range of the assay in various matrices was evaluated.

Sample Type	Average % Recovery	Range %
Cell culture media (n=5)	102.7	95.0-109.6
Serum (n=5)	99.8	94.9-106.8
Plasma (n=3)	89.8	82.8-95.6

C. SENSITIVITY

The minimum detectable dose (MDD) of mouse IL-6 is typically less than 0.62 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-6 produced at R&D Systems. The NIBSC Standard for Murine IL-6 (93/730), which was intended as a potency standard, was evaluated in this kit.

The dose response curve of the NIBSC Standard (93/730) parallels the Valukine standard curve. To convert sample values obtained with the Valukine Mouse IL-6 kit to approximate NIBSC 93/730 units, use the equation below.

NIBSC (93/730) approximate value (IU/mL) = 1.30 × Valukine Mouse IL-6 value (pg/mL)

E. LINEARITY

To assess the linearity of the assay, different samples were containing or spiked with high concentrations of mouse IL-6 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	101.1	92.0-110.2
1:4	101.5	90.9-113.9
1:8	99.4	90.6-113.0
1:16	98.3	88.5-113.5

F. SAMPLE VALUES

Cell Culture Supernates - Two spleen organ tissues from a mouse were homogenized and seeded in 100 mL of RPMI supplemented with 10% fetal bovine serum, 2 mM L-glutamine, 100 U/mL penicillin, 100 µg/mL streptomycin sulfate, and 10 µg/mL ConA for 2 days. The cell culture supernate was assayed for mouse IL-6 and measured 194 pg/mL.

EL-4 cells (mouse thymoma) were seeded at $2x10^5$ cell/mL and cultured for 4 days in 100 mL of DMEM supplemented with 10% horse serum, 10 µg/mL PHA and 10 ng/mL PMA. The cell culture supernate was assayed for mouse IL-6 and measured 317 pg/mL.

BALB/3T3 cells were seeded in 100 mL of DMEM supplemented with 10% fetal bovine serum for 2 days. The cell culture supernate was assayed for mouse IL-6 and measured 157.1 pg/mL.

Serum - Five serum samples were evaluated for the presence of IL-6 in this assay. All samples measured ranged from 45.7 to 94.2 pg/mL with an average of 65.7 pg/mL.

Plasma - Three plasma samples were evaluated for the presence of IL-6 in this assay. All samples measured ranged from 35.3 to 109.1 pg/mL with an average of 81.6 pg/mL.

G. SPECIFICITY

This assay recognizes both natural and recombinant mouse IL-6. 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-6 control were assayed for interference. No significant cross-reactivity or interference was observed.

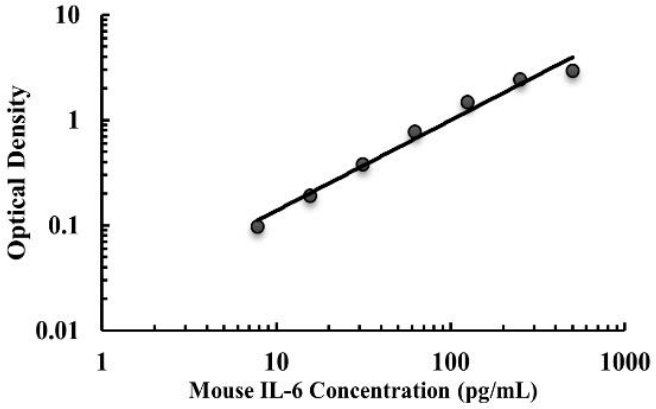
Recombinant Mouse	**Recombinant Human**
CT-1	IL-6
GP130	IL-6R
IL11	
LIF	
OSM	
IL-6R	

Recombinant Rat IL-6 cross-reacts approximately 0.24% in this assay.

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	0.053 0.053	0.053	-
7.8	0.152 0.147	0.149	0.097
15.6	0.242 0.247	0.245	0.192
31.3	0.435 0.433	0.434	0.381
62.5	0.826 0.817	0.822	0.769
125	1.522 1.526	1.524	1.471
250	2.500 2.456	2.478	2.425
500	2.987 2.986	2.987	2.934

V. KIT COMPONENTS AND STORAGE

A. MATERIALS PROVIDED

Parts	Description	Size
Mouse IL-6 Microplate	96 well polystyrene microplate (12 strips of 8 wells) coated with monoclonal antibody against mouse IL-6.	1 plate
Mouse IL-6 Detection Antibody	Biotinylated mouse IL-6 antibody, lyophilized. Refer to the vial label for reconstitution volume.	1 vial
Mouse IL-6 Standard	Recombinant mouse IL-6 in a buffered protein base, lyophilized. Refer to the vial label for reconstitution volume.	2 vials
Calibrator Diluent Concentrate (4×)	A 4× concentrated buffered protein base used to dilute standard, samples and Detection Antibody.	1 vial
Reagent Diluent Concentrate (10×)	A 10× concentrated buffered protein base used to dilute HRP.	1 vial
Streptavidin-HRP A (200×)	200× Streptavidin conjugated to horseradish peroxidase.	1 vial
Wash Buffer Concentrate (25×)	A 25× concentrated solution of buffered surfactant.	1 vial
TMB Substrate	TMB ELISA Substrate Solution.	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>Streptavidin-HRP A</td><td rowspan="4">May be stored for up to 1 month at 2-8 °C.*</td></tr>
<tr><td>Diluted Wash Solution</td></tr>
<tr><td>TMB Substrate</td></tr>
<tr><td>Stop Solution</td></tr>
<tr><td>Standard</td><td>Prepare fresh for each assay.</td></tr>
<tr><td>Detection Antibody</td><td>Aliquot and store for up to 1 month at -20 °C in a manual defrost freezer. *</td></tr>
<tr><td>Calibrator Diluent Concentrate (4×)</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>Reagent Diluent Concentrate (10×)</td><td>May be stored for up to 1 month at 2-8 °C.*
Use and discard diluted Reagent 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×).

Plasma - Collect plasma using EDTA, heparin, or citrate as an anticoagulant. Centrifuge for 15 minutes at 1000 x g within 30 minutes of collection. 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

Cell Culture Supernate, serum and plasma 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.

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

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

Detection Antibody - Centrifuge briefly before opening. Reconstitution volume refer to vial label with Calibrator Diluent (1×). Aliquot and store if needed. Dilute stock solution in Calibrator Diluent (1×) to the working concentration of 0.5 µg/mL. Prepare at least 15 minutes prior to use.

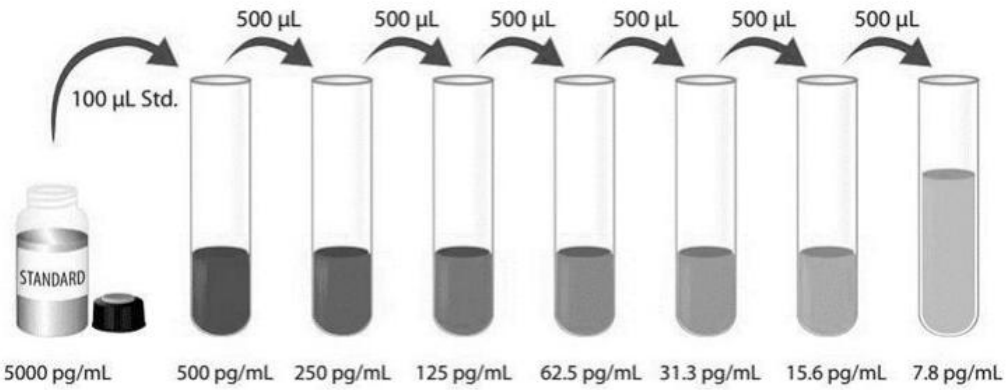
Streptavidin-HRP A (1×) - Centrifuge briefly before opening. Dilute to the working concentration specified on the vial label using Reagent Diluent (1×).

Mouse IL-6 Standard - Centrifuge briefly before opening. 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 the 500 pg/mL tube. Pipette 500 µL of the appropriate 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 500 pg/mL standard 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. Add 100 µL of standard, or prepared 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. (Samples may require dilution. See Sample Preparation section.)
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 100 µL of the Detection Antibody diluted in Calibrator Diluent (1×), to each well. Cover with a new adhesive strip and incubate 2 hours at room temperature.
6. Repeat the aspiration/wash as in step 4.
7. Add 100 µL of the working dilution of Streptavidin-HRP A to each well. Cover the plate and incubate for 30 minutes at room temperature. **Protect from light**.
8. Repeat the aspiration/wash as in step 4.
9. Add 100 µL of TMB Substrate to each well. Incubate for 20 minutes at room temperature. **Protect from light**.
10. Add 50 µL of Stop Solution to each well. Gently tap the plate to ensure thorough mixing.
11. Determine the optical density of each well 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.

12. **CALCULATION OF RESULTS.**

Average the duplicate readings for each standard 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 Mouse IL-6 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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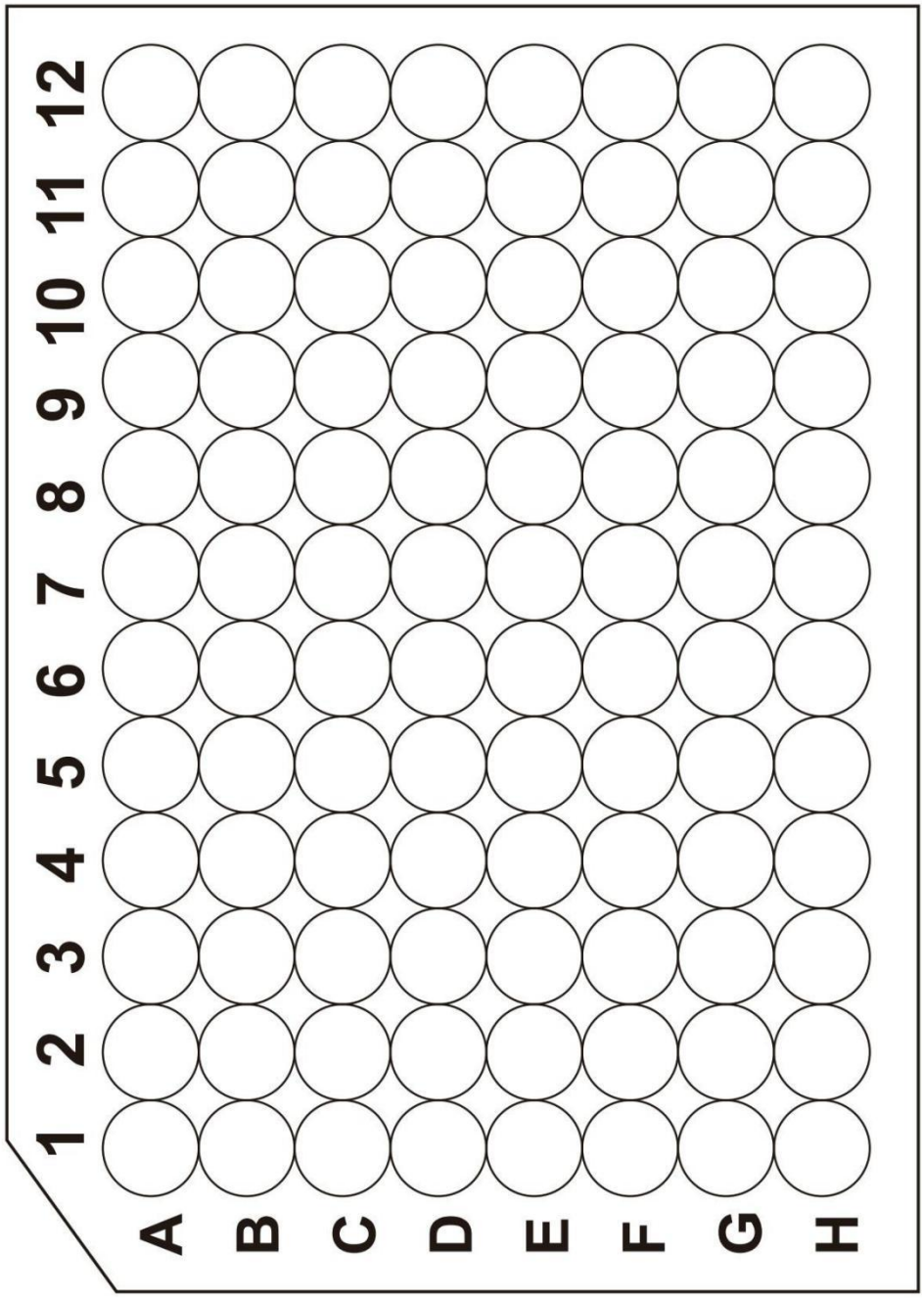
PLATE LAYOUT

Use this plate layout to record standards and samples assayed.



产品信息及操作手册

小鼠 IL-6 Valukine™ ELISA 试剂盒

目录号：VAL604G

适用于定量检测天然和重组小鼠 IL-6 的含量

科研专用，不可用于临床诊断

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版本号 202307.2

目录

I. 背景

白细胞介素-6（IL-6）是一个具有多效α螺旋结构，22-28kD的磷酸化和不同程度糖基化的多功能细胞因子，它在疾病急性期反应、炎症、造血、骨代谢以及癌症恶化等方面起重要作用（1-5）。成熟的小鼠IL-6有187个氨基酸，与人和大鼠IL-6分别有42%和85%同源性（6）。IL-6内部的选择性剪接而产生多种异构体，其中一些剪接体表现出拮抗特性（7-10）。已知表达IL-6的细胞，包括$CD8^+$ T细胞、成纤维细胞、滑膜细胞、脂肪细胞、成骨细胞、巨核细胞、内皮细胞（在内皮素的影响下）、交感神经元、大脑皮质神经细胞、肾上腺髓质嗜铬细胞、视网膜色素细胞、肥大细胞、角质形成细胞、朗格汉斯细胞、胎儿及成人星形胶质细胞、中性粒细胞、单核细胞、嗜酸性粒细胞，结肠上皮细胞、B1 B细胞和胰岛β细胞（2, 7, 10-33）。IL-6的产生通常与细胞活化相关，通常由糖皮质激素、儿茶酚胺和继发性类固醇控制（2）。正常人血液中的IL-6在1 pg/mL范围内，在月经期略有升高、在某些癌症有中度增高、在大型手术后有显著升高（34-38）。

IL-6通过由配体结合亚基IL-6R和信号转导亚基(gp130)组成的细胞表面异二聚体受体复合物诱导信号转导。IL-6与IL-6R结合，触发IL-6R与gp130的结合及gp130的二聚体化（39）。gp130也是CLC、CNTF、CT-1、IL-11、IL-27、LIF和OSM受体的组成部分（40）。可溶性IL-6R是由选择性剪接和蛋白酶切割产生的（3）。通过反式信号转导机制，可溶性IL-6和IL-6R复合物可引起表达gp130细胞的应答，这些细胞表面缺乏IL-6R（1, 3）。IL-6R的表达主要局限于肝细胞、单核细胞、淋巴细胞和静息淋巴细胞，由于gp130分子表达非常广泛，反式信号转导实现了更广泛的细胞类型对IL-6的响应（1-3）。可溶性gp130剪接体阻断了IL-6/IL-6R的反式信号转导，但不能阻断其它细胞因子利用gp130分子作为共同受体的信号转导（3, 41）。

IL-6与肿瘤坏死因子α（TNF-α）和IL-1一起驱动急性炎症反应，在发热和肝脏急性炎症反应中几乎起着独一无二的作用，它在急性炎症到获得性免疫或者慢性炎性疾病的转化中也发挥重要作用（1-4）。IL-6失调可促进慢性炎症，如肥胖、胰岛素抵抗、炎症性肠道疾病、炎性关节炎以及败血症，往往涉及 IL-6 的反式信号转导（1, 2）。在转化生长因子TGF-β存在的情况下，IL-6在幼稚型T细胞向Th17炎性细胞分化的过程中起重要作用。IL-6通过促进Th17 T细胞活性调节骨吸收，是类风湿性关节炎中造成炎性关节损伤的主要因素（1）。它有助于动脉粥样硬化斑块的形成和不稳定性（2）。然而，IL-6也有抗炎症作用，如运动锻炼时骨骼肌分泌IL-6（2），它作为造血干细胞的生长因子促进造血、诱导B细胞成熟为浆细胞、多发性骨髓瘤细胞的永生（1, 42）。IL-6也促进，但可能不会启动其它炎症相关的癌症发生，如结肠炎相关癌症（1）。

II. 概述

A. 检测原理

本实验采用双抗体夹心ELISA法。抗小鼠IL-6单抗包被于微孔板上，样品和标准品中的小鼠IL-6会与固定在板上的抗体结合，游离的成分被洗去；接着加入生物素化的抗小鼠IL-6检测抗体进行孵育，洗涤去除未结合的物质后，加入链霉亲和素标记的辣根过氧化物酶（streptavidin-HRP）孵育。洗涤去除未结合的试剂后，加入TMB底物溶液。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、小鼠血清和血浆；
- 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	3	1	2	3
平均值 (pg/mL)	245.8	62.4	15.2	247.7	61.9	15.1
标准差	10.4	2.8	0.7	9.7	2.6	0.6
CV%	4.2	4.5	4.5	3.9	4.2	4.0

B. 回收率

在不同类型样本中掺入检测范围内不同水平的小鼠IL-6，测定其回收率。

样本类型	平均回收率%	范围%
细胞培养上清 (n=5)	102.7	95.0-109.6
血清 (n=5)	99.8	94.9-106.8
血浆 (n=3)	89.8	82.8-95.6

C. 灵敏度

小鼠IL-6的最低可测剂量（MDD）一般小于0.62 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R&D Systems生产的大肠杆菌表达的高纯度重组小鼠IL-6蛋白所校正。

NIBSC鼠IL-6标准品(93/730)作为效价标准，在本试剂盒中进行了评估。NIBSC标准品(93/730)的剂量反应曲线与Valukine标准曲线平行。若要将使用Valukine Mouse IL-6 kit

获得的样本值转换为NIBSC 93/730的近似单位，请使用以下公式：

NIBSC (93/730) approximate value (IU/mL) = 1.30 × Valukine Mouse IL-6 value (pg/mL)

E. 线性

不同的样本中含有或掺入高浓度的小鼠 IL-6，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	101.1	92.0-110.2
1:4	101.5	90.9-113.9
1:8	99.4	90.6-113.0
1:16	98.3	88.5-113.5

F. 样本预值

细胞培养上清液 - 从两个小鼠脾脏组织匀浆，得到的原代细胞培养于100 mL的RPMI培养基中，细胞培养基还含有10%胎牛血清、2 mM L-谷氨酰氨、100 U/mL青霉素、100 µg/mL硫酸链霉素，10 µg/mL Con A，培养两天。取细胞培养上清测定小鼠IL-6含量，结果为194 pg/mL。

EL-4细胞（小鼠胸腺瘤），以2× 10^5细胞/mL培植，培养四天：培养基为100 mL DMEM含有10%马血清、10 µg/mL PHA和10 ng/mL PMA。取细胞培养上清测定小鼠IL-6含量，结果为317 pg/mL。

BALB/3T3培养于100 mL的DMEM培养基中，细胞培养基还含有10%胎牛血清，培养两天。取细胞培养上清测定小鼠IL-6含量，结果为157.1 pg/mL。

血清样本 - 使用本试剂盒检测了5份小鼠血清样本中IL-6的水平。5份样本的检测值在45.7- 94.2 pg/mL之间，平均值为65.7 pg/mL。

血浆样本 - 使用本试剂盒检测了3份小鼠血清样本中IL-6的水平。3份样本的检测值在35.3 - 109.1 pg/mL之间，平均值为81.6 pg/mL。

G. 特异性

此ELISA法可检测天然及重组小鼠IL-6蛋白。将以下因子用标准品稀释液（1×）配制成50 ng/mL的浓度来检测与小鼠IL-6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-6对照品中，来检测对小鼠IL-6的干扰。没有观察到明显的交叉反应或干扰。

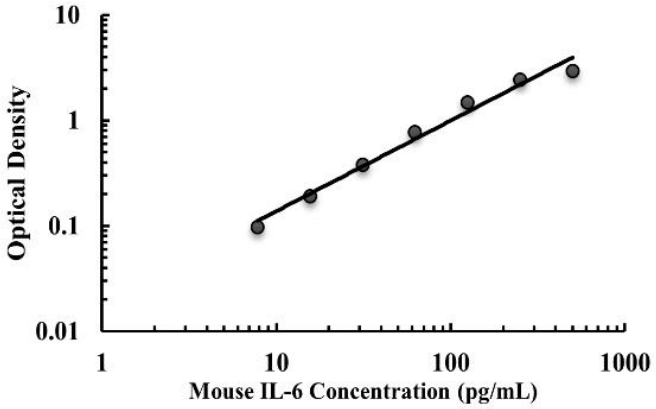
Recombinant Mouse	**Recombinant Human**
CT-1	IL-6
GP130	IL-6R
IL11	
LIF	
OSM	
IL-6R	

重组大鼠IL-6在此试剂盒中有0.24%的交叉反应。

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	0.053 0.053	0.053	-
7.8	0.152 0.147	0.149	0.097
15.6	0.242 0.247	0.245	0.192
31.3	0.435 0.433	0.434	0.381
62.5	0.826 0.817	0.822	0.769
125	1.522 1.526	1.524	1.471
250	2.500 2.456	2.478	2.425
500	2.987 2.986	2.987	2.934

V. 试剂盒组成及储存

A. 试剂盒组成

组成	描述	规格
Mouse IL-6 Microplate	包被抗体的96孔聚苯乙烯板，8孔×12条	1块板
Mouse IL-6 Detection Antibody	生物素化的小鼠IL-6检测抗体，冻干粉，参考瓶身标签进行重溶	1瓶
Mouse IL-6 Standard	标准品（冻干），参考瓶身标签进行重溶	2瓶
Calibrator Diluent Concentrate (4×)	浓缩标准品稀释液（4×）用于稀释样本、标准品和检测抗体	1瓶
Reagent Diluent Concentrate (10×)	浓缩的试剂稀释液（10×）用于稀释HRP	1瓶
Streptavidin-HRP A (200×)	200×浓缩的链霉亲和素标记的HRP A	1瓶
Wash Buffer Concentrate (25×)	浓缩洗涤缓冲液（25×）	1瓶
TMB Substrate	TMB ELISA底物溶液	1瓶
Stop Solution	终止液	1瓶
Plate Covers	封板膜	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>链霉亲和素-HRP A</td><td rowspan="4">2-8℃储存，最多 30 天*</td></tr>
<tr><td>洗涤缓冲液（1×）</td></tr>
<tr><td>TMB 底物溶液</td></tr>
<tr><td>终止液</td></tr>
<tr><td>标准品</td><td>使用时新鲜配制*</td></tr>
<tr><td>检测抗体</td><td>分装， -20℃储存，最多 30 天*</td></tr>
<tr><td>标准品稀释液（4×）</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>试剂稀释液（10×）</td><td>2-8℃储存，最多 30 天*
请每次使用新鲜配制的 1×试剂稀释液，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。

血浆样本: 使用EDTA、肝素钠或枸橼酸钠作为抗凝剂收集血浆。然后1000 x g离心15分钟，需在30分钟内收集血浆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

细胞培养上清、血清和血浆样本需要用标准品稀释液（1×）2倍稀释后进行检测，即100 µL样本+100 µL标准品稀释液（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制成500 mL工作浓度的洗涤液。

标准品稀释液（1×）：使用去离子水或蒸馏水稀释配制成标准品稀释液（1×）。

试剂稀释液（1×）：使用去离子水或蒸馏水稀释配制成试剂稀释液（1×）。

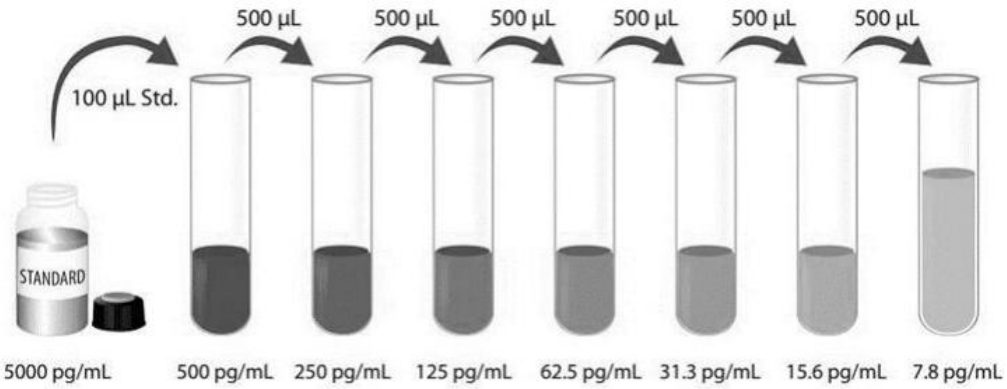
检测抗体：开盖前请瞬时离心。参考检测抗体瓶标签指示，用标准品稀释液（1×）将冻干粉进行重溶。再用标准品稀释液（1×）稀释至工作浓度0.5 µg/mL，至少在使用前15分钟准备。

链霉亲和素- HRP A（1×）：开盖前请瞬时离心。用试剂稀释液（1×）将链霉亲和素- HRP A（200×）稀释至工作浓度链霉亲和素- HRP A（1×）。

标准品：开盖前请瞬时离心。参照标准品瓶身注明的方式重溶冻干标准品。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。

500 pg/mL稀释管中加入900 uL标准品稀释液（1×），其余各稀释管中加入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×）可用作标准品零点（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，实验样本或者质控品加入相应孔中，每孔100 µL。用封板膜封住反应孔，室温孵育2小时。说明书提供了一张96孔模板图，可用于记录标准品和试验样本的板内位置；（样本需要稀释，详情参见样本制备部分。）
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检测抗体。用封板膜封住反应孔，室温孵育2小时；
6. 重复第4步洗板操作；
7. 在每个微孔内加入100 µL稀释好的链霉亲和素- HRP A工作液。用封板膜封住反应孔，室温孵育30分钟，**注意避光**；
8. 重复第4步洗板操作；
9. 在每个微孔内加入100 µL TMB底物溶液，室温孵育20分钟。**注意避光**；
10. 在每个微孔内加入50 µL终止液，请轻拍微孔板，使溶液混合均匀；
11. 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；
12. **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-6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