# PRODUCT INFORMATION & MANUAL

## Human IL-6 Valukine™ ELISA

## VAL102

For the quantitative determination of natural and recombinant human Interleukin 6 (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

Version202209.5

# TABLE OF CONTENTS

# I. BACKGROUND

Interleukin 6 (IL-6) is a pleiotropic α-helical 22-28 kDa phosphorylated and variably glycosylated cytokine that plays important roles in the acute phase reaction, inflammation, hematopoiesis, bone metabolism, and cancer progression (1-5). Mature human IL-6 is 183 amino acids (aa) in length and shares 41% aa sequence identity with mouse and rat IL-6 (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 endothelins), 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-6 R) and a signal transducing subunit (gp130). IL-6 binds to IL-6 R, triggering IL-6 R 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-6 R are generated by both alternative splicing and proteolytic cleavage (3). In a mechanism known as trans-signaling, complexes of soluble IL-6 and IL-6 R elicit responses from gp130-expressing cells that lack cell surface IL-6 R (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-6 R is predominantly restricted to hepatocytes, monocytes, and resting lymphocytes (1-3). Soluble splice forms of gp130 block trans-signaling from IL-6/IL-6 R 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 IL-6 has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any IL-6 present is bound by the immobilized antibody. After washing away any unbound substances, an enzyme-linked polyclonal antibody specific for IL-6 is added to the wells. Following a wash to remove any unbound antibody-enzyme reagent, a substrate solution is added to the wells and color develops in proportion to the amount of IL-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, 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 Diluent and repeat the assay.
- Any variation in operator, pipetting technique, washing technique, incubation time or temperature, and kit age can cause variation in binding.

# III. ADVANTAGES

## A. PRECISION

**Intra-assay Precision** (Precision within an assay)

Three samples were tested twenty times on one plate to assess intra-assay precision.

**Inter-assay Precision** (Precision between assays)

Three samples were tested in twenty separate assays to assess inter-assay precision.

| | Intra-assay Precision | | | Inter-assay Precision | | |
|---|---|---|---|---|---|---|
| Sample | 1 | 2 | 3 | 1 | 2 | 3 |
| Mean (pg/mL) | 20.6 | 77.8 | 175 | 23.9 | 83.3 | 177 |
| Standard Deviation | 1.20 | 3.51 | 7.33 | 4.20 | 13.3 | 25.5 |
| CV% | 5.8 | 4.5 | 4.2 | 17.6 | 15.9 | 14.4 |

## B. RECOVERY

The recovery of human 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=4) | 95 | 81 - 104% |
| Serum (n=3) | 93 | 80 - 99% |
| Plasma (n=4) | 96 | 81 - 109% |

## C. SENSITIVITY

The minimum detectable dose (MDD) of IL-6 is typically less than 1.56 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 human IL-6 produced at R&D Systems. The NIBSC/WHO 1st International Standard for IL-6 (89/548), which was intended as a potency standard, was evaluated in this kit. The NIBSC/WHO standard is a CHO cell-derived recombinant human IL-6.

The dose response curve of the International Standard (89/548) parallels the Valukine standard curve. To convert sample values obtained with the Valukine Human IL-6 kit to approximate NIBSC 89/548 units, use the equation below.

NIBSC (89/548) approximate value (IU/mL) = 0.109 × Valukine Human IL-6 value (pg/mL)

## E. LINEARITY

To assess the linearity of the assay, samples were spiked with high concentrations of human IL-6 in various matrices and diluted with Diluent 1× to produce samples with values within the dynamic range of the assay.

| Dilution | | Cell culture media (n=4) | Serum (n=3) | Plasma (n=4) |
|---|---|---|---|---|
| 1:2 | Average % of Expected | 112 | 102 | 99 |
| | Range (%) | 105 - 117 | 101 - 102 | 89 - 106 |
| 1:4 | Average % of Expected | 111 | 106 | 101 |
| | Range (%) | 104 - 119 | 102 - 111 | 95 - 107 |
| 1:8 | Average % of Expected | 97 | 108 | 99 |
| | Range (%) | 91 - 105 | 103 - 116 | 93 - 104 |
| 1:16 | Average % of Expected | 89 | 109 | 98 |
| | Range (%) | 81 - 98 | 102 - 117 | 90 - 107 |

## F. SAMPLE VALUES

**Cell Culture Supernates -** Human peripheral blood mononuclear cells ($1\times10^6$ 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 and stimulated for 3 days with 10 µg/mL PHA. An aliquot of the cell culture supernate was removed, assayed for levels of natural IL-6, and measured 6640 pg/mL.

**Serum -** Three human serum samples were evaluated for the presence of human IL-6 in this assay. All samples measured ranged from 20.5 to 62.5 pg/mL with an average of 48.0 pg/mL.

**Plasma -** Four human plasma samples were evaluated for the presence of human IL-6 in this assay. All samples measured ranged from 73.5 to 105 pg/mL with an average of 88.6 pg/mL.

## G. SPECIFICITY

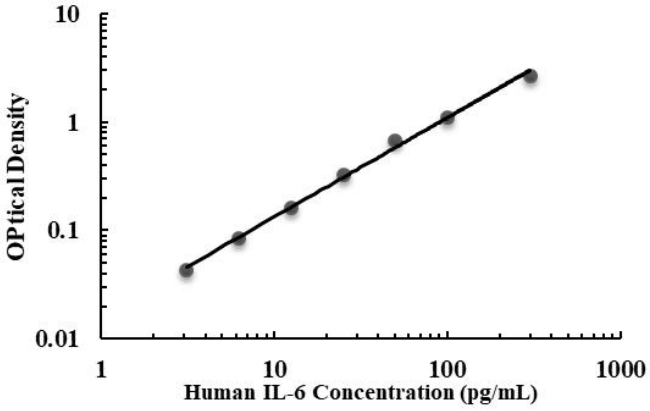
This assay recognizes both natural and recombinant human IL-6. The following factors were prepared at 50 ng/mL and assayed for cross-reactivity. Preparations of the following factors at 50 ng/mL in a mid-range rhIL-6 control were assayed for interference. No significant cross-reactivity or interference was observed.

| Recombinant human | Recombinant mouse |
|---|---|
| sgp130 | IL-6 |
| IL-6 sR | |
| IL-6 sR/sgp130 | |

# 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 | 0.024<br>0.022 | 0.023 | - |
| 3.12 | 0.067<br>0.065 | 0.066 | 0.043 |
| 6.25 | 0.108<br>0.105 | 0.107 | 0.084 |
| 12.5 | 0.190<br>0.180 | 0.185 | 0.162 |
| 25 | 0.355<br>0.343 | 0.349 | 0.326 |
| 50 | 0.714<br>0.682 | 0.698 | 0.675 |
| 100 | 1.110<br>1.141 | 1.126 | 1.103 |
| 300 | 2.629<br>2.808 | 2.719 | 2.696 |

# V. KIT COMPONENTS AND STORAGE

## A. MATERIALS PROVIDED

| Parts | Description | Size |
|---|---|---|
| Human IL-6 Microplate | 96 well polystyrene microplate (12 strips of 8 wells) coated with a mouse monoclonal antibody against human IL-6 | 1 plate |
| Human IL-6 Conjugate | Solution of polyclonal antibody against human IL-6 conjugated to horseradish peroxidase | 1 vial |
| Human IL-6 Standard | recombinant human IL-6 in a buffered protein base; lyophilized | 1 vial |
| Calibrator Diluent (5×) | a 5× concentrated buffered protein base | 1 vial |
| Wash Buffer Concentrate (25×) | a 25× concentrated solution of buffered surfactant | 1 vial |
| TMB Substrate | TMB ELISA 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/<br>Reconstituted<br>Reagents</td><td>Diluted Wash Buffer</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>Diluent 1×</td></tr>
<tr><td>Conjugate</td></tr>
<tr><td>TMB Substrate</td><td rowspan="2">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>Standard</td></tr>
<tr><td></td><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.

**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.

## B. SAMPLE PREPARATION

Serum samples require a 5-fold dilution. A suggested 5-fold dilution is 40 µL of sample + 160 µL of Diluent (1×).

Plasma samples require a 2-fold dilution. A suggested 2-fold dilution is 100 µL of sample + 100 µL of Diluent (1×).

## C. REAGENT PREPARATION

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

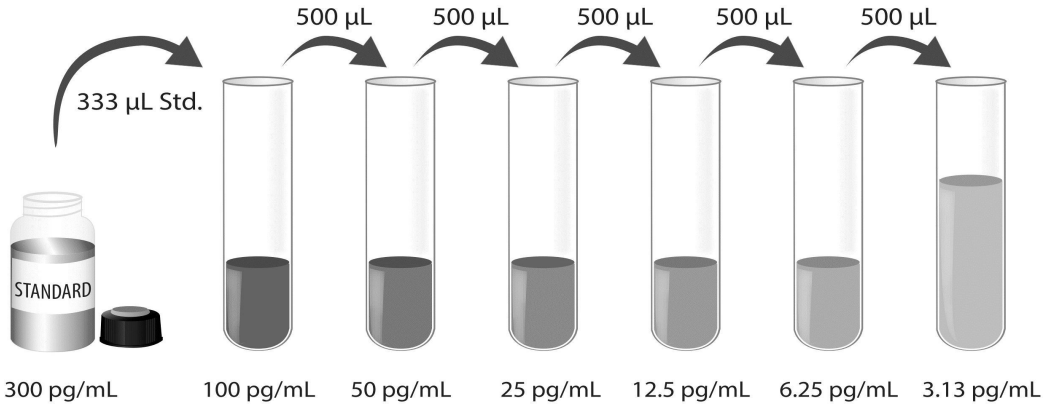
**Wash Buffer -** If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. Dilute 20 mL of Wash Buffer Concentrate (25×) into deionized or distilled water to prepare 500 mL of Wash Buffer.

**Diluent 1× -** Add 20 mL of Calibrator Diluent Concentrate 5× into 80 mL of deionized or distilled water to prepare 100 mL of Diluent 1×.

**IL-6 Standard - Refer to the vial label for reconstitution volume*.** This reconstitution produces a stock solution of 300 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 667 µL of Diluent 1× into the 100 pg/mL tube. Pipette 500 µL of 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 undiluted standard serves as the high standard (300 pg/mL). The 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 Substrate Solution protected from light. Substrate Solution should change from colorless to gradations of blue.
- Stop Solution should be added to the plate in the same order as the Substrate Solution. The color developed in the wells will turn from blue to yellow upon addition of the Stop Solution. Wells that are green in color indicate that the Stop Solution has not mixed thoroughly with the Substrate Solution.

## VII.ASSAY PROCEDURE

***Note:*** Bring all reagents and samples to room temperature before use. It is recommended that all samples and standards be assayed in duplicate.

1. Prepare all reagents and working standards as directed in the previous sections.
2. Remove excess microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, and reseal.
3. Add 100 µL of Standard, sample, or control per well. Cover with the adhesive strip provided. Incubate for 2 hours at room temperature. A plate layout is provided for a record of standards and samples assayed.
4. Aspirate each well and wash, repeating the process three times for a total of four washes. Wash by filling each well with Wash 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 human IL-6 Conjugate to each well. Cover with a new adhesive strip. Incubate for 2 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 20 minutes at room temperature. **Protect from light.**
8. Add 50 µL of Stop Solution to each well. The color in the wells should change from blue to yellow. If the color in the wells is green or if the color change does not appear uniform, gently tap the plate to ensure thorough mixing.
9. Determine the optical density of each well within 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, 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-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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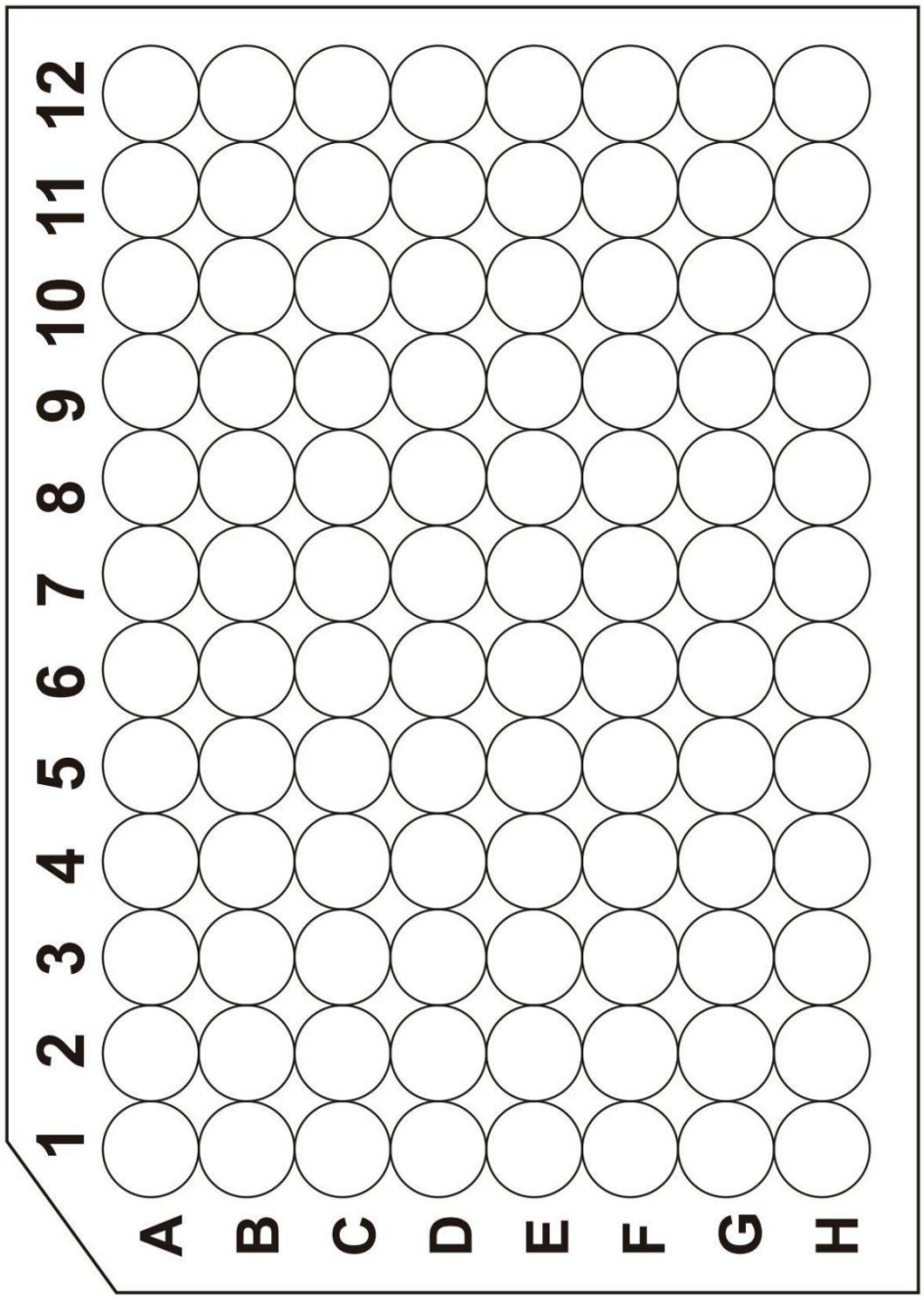
## PLATE LAYOUT

Use this plate layout to record standards and samples assayed.



# 产品信息及操作手册

## 人 IL-6 Valukine™ ELISA 试剂盒

## 目录号：VAL102

适用于定量检测天然和重组人白介素 6（IL-6）的浓度

科研专用，不可用于临床诊断

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# 目录

# I. 背景

白细胞介素-6（IL-6）是一个具有α螺旋结构、22-28 kDa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）。成熟的人IL-6有183个氨基酸，与小鼠和大鼠IL-6有41%的同源性（6）。IL-6内部的选择性剪接而产生多种异构体，有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、胎儿和成人星形胶质细胞、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的产生通常是在糖皮质激素、儿茶酚胺和第二性类固醇控制之下，一般与细胞激活相关（2）。正常人血液中的IL-6在1pg/mL范围内，在月经期略有升高、在某些癌症中有中度增高、在手术后有显著提高（34-38）。

IL-6通过细胞表面异二聚体受体复合物引发细胞信号，该复合物由一个配体结合亚单位（IL-6受体）和一个信号转导亚单位gp130组成。IL-6结合到IL-6受体上，引发了IL-6受体与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-6受体是由选择性剪接和蛋白水解产生的（3）。通过一个反式信号传导机制，可溶性IL-6和IL-6受体复合物可引发表面缺乏IL-6受体但表达gp130细胞的应答（1, 3）。IL-6受体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剪接体阻止了IL-6/IL-6R的反式信号转导，但不能阻止其他细胞因子利用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-1一起所引起的急性炎症反应，在发热和肝脏急性炎症反应中几乎起着独一无二的作用，它在急性炎症转化为获得性免疫或者慢性炎症疾病时也发挥重要作用（1-4）。IL-6失调有助于如肥胖、胰岛素抵抗、炎症性肠道疾病、炎性关节炎以及败血症情况下的慢性炎症，往往涉及IL-6的反式信号转导（1, 2）。在转化生长因子 TGF-β存在的条件下，IL-6在幼稚型T细胞向Th17炎性细胞分化的过程中其中起重要作用。IL-6调节骨吸收，并且通过促进Th17炎性细胞的活性，成为类风湿性关节炎中造成炎性关节损伤的主要因素（1）。IL-6有助于动脉粥样硬化斑块的形成和不稳定性（2）。但是IL-6也有抗炎症作用，如体育锻炼时骨骼肌分泌IL-6（2）。它作为造血干细胞生长因子促进造血、诱导B细胞成熟为浆细胞、永生化多发性骨髓瘤细胞（1, 42）。IL-6也促进但是启动其它炎症相关的癌症发生，如肠炎相关癌（1）。

# II. 概述

## A. 检测原理

本实验采用双抗体夹心ELISA法。抗人IL-6单抗包被于微孔板上，样品和标准品中的IL-6会与固定在板上的抗体结合，游离的成分被洗去；加入辣根过氧化酶标记的抗人IL-6多抗，与结合在微孔板上的IL-6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，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、人血清和血浆样本；
- 请在有效期内使用试剂盒；
- 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) | 20.6 | 77.8 | 175 | 23.9 | 83.3 | 177 |
| 标准差 | 1.20 | 3.51 | 7.33 | 4.20 | 13.3 | 25.5 |
| CV% | 5.8 | 4.5 | 4.2 | 17.6 | 15.9 | 14.4 |

### B. 回收率

在不同类型样本中掺入检测范围内不同水平的人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=4) | 95 | 81 - 104% |
| 血清 (n=3) | 93 | 80 - 99% |
| 血浆 (n=4) | 96 | 81 - 109% |

### C. 灵敏度

人IL-6的最低可测剂量（MDD）一般小于1.56pg/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试剂盒是针对R&D Systems生产的大肠杆菌表达的高纯度重组人IL-6进行校准的。NIBSC/WHO IL-6第1国际标准品(89/548)作为效价标准，在本试剂盒中进行了评估。NIBSC/WHO标准品是CHO细胞来源的重组人IL-6。

国际标准品(89/548)的剂量反应曲线与Valukine标准曲线平行。若要将使用Valukine Human IL-6 kit获得的样本值转换为NIBSC 89/548的近似单位，请使用以下公式：

NIBSC (89/548) approximate value (IU/mL) = 0.109 × Valukine Human IL-6 value (pg/mL)

## E. 线性

在不同类型样本中掺入高浓度的人IL-6，然后用稀释剂将样本稀释到检测范围内，测定其线性。

| 稀释倍数 | | 细胞培养上清（n=4） | 血清（n=3） | 血浆（n=4） |
|---|---|---|---|---|
| 1:2 | 平均值/期待值（%） | 112 | 102 | 99 |
| | 范围（%） | 105 - 117 | 101 - 102 | 89 - 106 |
| 1:4 | 平均值/期待值（%） | 111 | 106 | 101 |
| | 范围（%） | 104 - 119 | 102 - 111 | 95 - 107 |
| 1:8 | 平均值/期待值（%） | 97 | 108 | 99 |
| | 范围（%） | 91 - 105 | 103 - 116 | 93 - 104 |
| 1:16 | 平均值/期待值（%） | 89 | 109 | 98 |
| | 范围（%） | 81 - 98 | 102 - 117 | 90 - 107 |

## F. 样本预值

细胞上清样本 -人的外周血单核细胞（1×$10^6$细胞/mL）培养于含有10%胎牛血清的RPMI1640 培养基中，细胞培养基还含有2mM L-谷氨酰胺、50μM β-巯基乙醇、100U/mL青霉素、100μg/mL硫酸链霉素、10μg/mL PHA刺激细胞，培养3天。取细胞培养上清液测定IL-6含量， 结果为6640 pg/mL。

血清样本 -使用本试剂盒检测了3份人血清样本中IL-6的水平。3份样本的检测值在20.5-62.5pg/mL之间，平均值为48.0pg/mL。

血浆样本 - 使用本试剂盒检测了4份人血浆样本中IL-6的水平。4份样本的检测值在73.5 - 105pg/mL之间，平均值为88.6pg/mL。

## G. 特异性

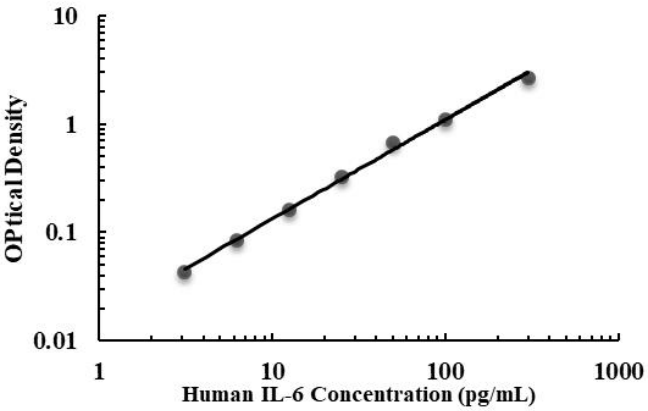
此ELISA法可检测天然及重组人IL-6蛋白。将以下因子用稀释剂（1×）配置成50ng/mL的浓度来检测与人IL-6的交叉反应。将50ng/mL的干扰因子掺入到中间范围的重组人IL-6控制品中，来检测对人IL-6的干扰。没有观察到明显的交叉反应或干扰。

| 重组人蛋白 | 重组小鼠蛋白 |
| --- | --- |
| sgp130 | IL-6 |
| IL-6 sR | |
| IL-6 sR/sgp130 | |

# 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 | 0.024<br>0.022 | 0.023 | - |
| 3.12 | 0.067<br>0.065 | 0.066 | 0.043 |
| 6.25 | 0.108<br>0.105 | 0.107 | 0.084 |
| 12.5 | 0.190<br>0.180 | 0.185 | 0.162 |
| 25 | 0.355<br>0.343 | 0.349 | 0.326 |
| 50 | 0.714<br>0.682 | 0.698 | 0.675 |
| 100 | 1.110<br>1.141 | 1.126 | 1.103 |
| 300 | 2.629<br>2.808 | 2.719 | 2.696 |

# V. 试剂盒组成及储存

## A. 试剂盒组成

| 组成 | 描述 | 规格 |
|---|---|---|
| 人IL-6 Microplate | 包被抗体的96孔聚苯乙烯板，8孔×12条 | 1块板 |
| 人IL-6 Conjugate | 酶标人IL-6检测抗体 | 1瓶 |
| 人IL-6 Standard | 标准品（冻干） | 1瓶 |
| Calibrator Diluent（5×） | 浓缩稀释剂（5×） | 1瓶 |
| Wash Buffer Concentrate（25×） | 浓缩洗涤缓冲液（25×） | 1瓶 |
| TMB Substrate | TMB底物溶液 | 2瓶 |
| Stop Solution | 终止液 | 1瓶 |
| Plate Sealers | 封板胶纸 | 3张 |

## B. 试剂盒储存

<table>
<tr><td>未开封试剂盒</td><td colspan="2">2-8℃储存；请在试剂盒有效期内使用</td></tr>
<tr><td rowspan="6">已打开，稀释或重溶的试剂</td><td>洗涤缓冲液（1×）</td><td rowspan="4">2-8℃储存，最多30天*</td></tr>
<tr><td>终止液</td></tr>
<tr><td>稀释剂（1×）</td></tr>
<tr><td>酶标检测抗体</td></tr>
<tr><td>TMB底物溶液</td><td rowspan="2">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</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450nm检测波长的吸收值及540nm或570nm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
- 500mL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1000xg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。

**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1000xg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。

## B. 样本准备工作

血清样本需要用稀释剂（1×）5倍稀释后进行检测，即40μL血清+160μL稀释剂（1×）。

血浆样本需要用稀释剂（1×）2倍稀释后进行检测，即100μL血浆+100μL稀释剂（1×）。

## C. 检测前准备工作

**使用前请将所有试剂放置于室温**

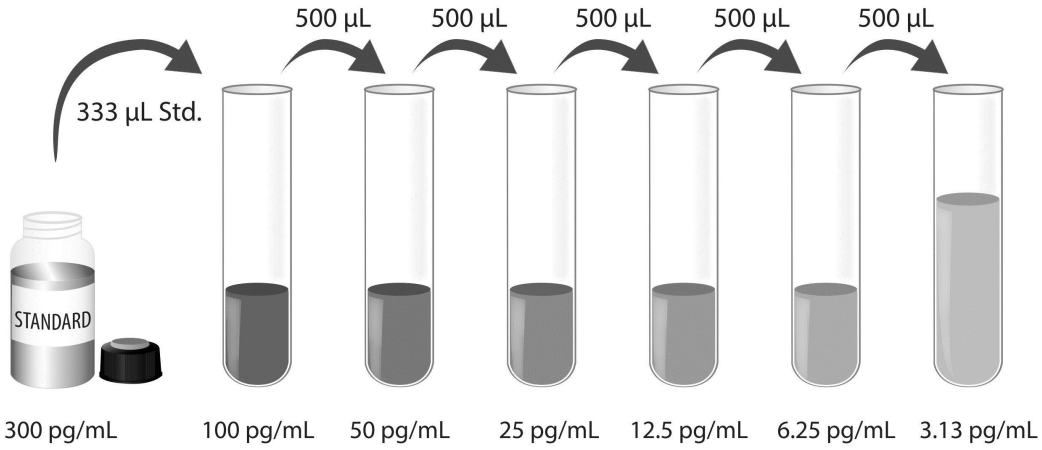
洗涤液：从冰箱中取出的浓缩洗涤液可能有结晶，属于正常现象；放置室温，轻摇混匀，待结晶完全溶解后再配制洗涤液。可将20mL浓缩洗涤液用蒸馏水或去离子水稀释配置成500mL工作浓度的洗涤液。未用完的放回4℃。

**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100mL工作浓度的稀释剂。

**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300pg/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100pg/mL稀释管中加入667μL稀释剂（1×），其余每个稀释管中加入500μL稀释剂（1×）。将标准品母液参照下图做系列稀释，每管须充分混匀后再移液到下一管。没有稀释的标准品母液可用作标准曲线最高点（300pg/mL），稀释剂（1×）可用作标准曲线零点（0pg/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；
- 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. 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100μL。用封板胶纸封住反应孔，室温孵育2小时。说明书提供了一张96孔模板图，可用于记录标准品和试验样本的板内位置；
4. 将板内液体吸去，使用洗瓶、多通道洗板器或自动洗板机洗板。每孔加洗涤液400μL，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200μL酶标检测抗体。用封板胶纸封住反应孔，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200μL TMB底物溶液，室温孵育20分钟。注意避光；
8. 在每个微孔内加入50μL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10分钟内，使用酶标仪测量450nm的吸光度值，设定540nm或570nm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