PRODUCT INFORMATION & MANUAL

Human IL-6 Valukine™ ELISA

Catalog Number: VAL102C

For the quantitative determination of natural and recombinant human Interleukin 6 (IL-6) concentrations

For research use only.
Not for diagnostic or therapeutic procedures.

Bio-Techne China Co. Ltd
P: +86 (21) 52380373 P: 8009881270 F: +86 (21) 52381001
info.cn@bio-techne.com

Please refer to the kit label for expiry date.
Novus kits are guaranteed for 3 months from date of receipt

Version 202604.2

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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 39% 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, 11-33). 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 alpha) 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. An antibody specific for human IL-6 has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any human IL-6 present is bound by the immobilized antibody. After washing away any unbound substances, an enzyme-linked antibody specific for human IL-6 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 human 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, human serum, human plasma and human urine.
- 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 RD5-3 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)	38.8	94.7	205	31.8	97.4	191
Standard Deviation	0.687	2.02	4.69	1.35	3.65	8.25
CV%	1.8	2.1	2.3	4.2	3.8	4.3

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 supernate (n=4)	102	82 - 122
Human serum (n=4)	96	89 - 103
Human EDTA plasma (n=4)	97	90 - 102
Human heparin plasma (n=4)	96	90 - 99
Human urine (n=4)	97	89 - 101

C. SENSITIVITY

Twenty-four assays were evaluated and the minimum detectable dose (MDD) of human IL-6 ranged from 0.120-0.626 pg/mL. The mean MDD was 0.368 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 a highly purified recombinant human IL-6 produced at R&D Systems.

The NIBSC/WHO Interleukin-6 International Standard 89/548 (IL-6, Human rDNA derived) was evaluated in this kit. 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) =0.1006 × Valukine Human IL-6 value (pg/mL)

Additionally, the 2nd International NIBSC/WHO Standard for Interleukin-6 21/308 (IL-6, Human recombinant) was evaluated in this kit. The dose response curve of the International Standard (21/308) parallels the Valukine standard curve. To convert sample values obtained with the Valukine Human IL-6 kit to approximate NIBSC 21/308 units, use the equation below.

NIBSC (21/308) approximate value (IU/mL) =0.0978 × Valukine Human IL-6 value (pg/mL)

Based on data generated from April 2023.

E. LINEARITY

To assess the linearity of the assay, samples were containing or spiked with high concentrations of human IL-6 in various matrices and diluted with Calibrator Diluent RD5-3 to produce samples with values within the dynamic range of the assay.

Dilution		Cell culture supernate (n=3)	Human serum (n=4)	Human EDTA Plasma (n=4)	Human heparin plasma (n=4)	Human urine (n=4)
1:2	Average % of Expected	97	104	103	105	100
	Range (%)	81-109	94-112	95-111	98-111	98-105
1:4	Average % of Expected	100	106	105	106	101
	Range (%)	88-116	97-115	95-115	98-115	98-104
1:8	Average % of Expected	107	108	107	110	100
	Range (%)	95-121	99-118	99-115	102-120	98-103
1:16	Average % of Expected	116	109	108	109	101
	Range (%)	99-125	103-119	101-116	99-117	98-105

F. SAMPLE VALUES

Human serum/plasma- Samples from apparently healthy volunteers were evaluated for the presence of human IL-6 in this assay. No medical histories were available for the donors used in this study.

Sample Type	Mean of Detectable (pg/mL)	%Detectable	Range (pg/mL)
Human serum (n=30)	6.90	40	ND-13.9
Human EDTA plasma (n=30)	6.82	40	ND-13.4
Human heparin plasma (n=30)	6.89	40	ND-12.9

ND=Non-detectable

Cell culture supernates - Human peripheral blood mononuclear cells (1 x 10^6 cells/mL) were cultured in RPMI supplemented with 10% fetal bovine serum, 50 µM β-mercaptoethanol, 2 mM L-glutamine, 100 units/mL penicillin, and 100 µg/mL streptomycin sulfate. PBMCs were left untreated or treated with 10 ug/mL PHA for 5 days before collecting conditioned media. Aliquots of the cell culture supernates were removed and assayed for levels of human IL-6.

Condition	Day 5 (pg/mL)
Unstimulated	24.0
Stimulated	10,986

Human urine - Ten samples from apparently healthy volunteers were evaluated for the presence of human IL-6 in this assay. No medical histories were available for the donors used in this study. All samples measured less than the lowest human IL-6 standard, 3.13 pg/mL.

G. SPECIFICITY

This assay recognizes natural and recombinant human IL-6.

The factors listed below were prepared at 3 ng/mL in calibrator diluent RD5-3 and assayed for cross-reactivity. Preparations of the following factors at 3 ng/mL in a mid-range recombinant human IL-6 control were assayed for interference. No significant cross-reactivity or interference was observed.

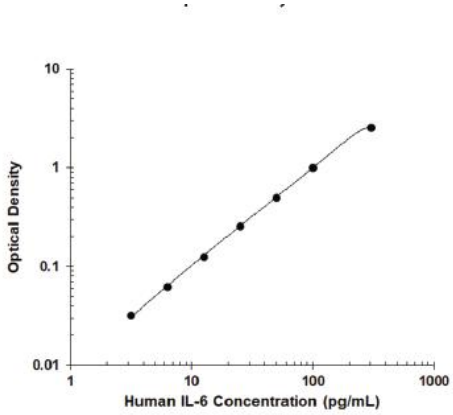
Recombinant human:	Other recombinants:
gp130	mouse IL-6
IL-6 Rα/gp130	rat IL-6
IL-6 sR	porcine IL-6
IL-6 R	

Recombinant cyno macaque IL-6 cross-reacts at approximately 79% and interferes at levels > 50 pg/mL.

IV. EXPERIMENT

EXAMPLE STANDARD

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



(pg/mL)	O.D.	Average	Corrected
0	0.005 0.005	0.005	—
3.13	0.032 0.032	0.032	0.027
6.25	0.063 0.063	0.063	0.058
12.5	0.124 0.125	0.125	0.120
25	0.255 0.263	0.259	0.254
50	0.504 0.504	0.504	0.499
100	0.987 1.010	0.999	0.994
300	2.569 2.575	2.572	2.567

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 an antibody against human IL-6	1 plate
Human IL-6 Conjugate	Solution of 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. Refer to the vial label for reconstitution volume.	2 vials
Assay Diluent RD1W	A buffered protein base with preservatives	1 vial
Calibrator Diluent RD5-3	A buffered protein base used to dilute standard and samples	1 vial
Wash Buffer Concentrate (25×)	A 25× concentrated solution of buffered surfactant	1 vial
TMB Substrate	TMB ELISA Substrate Solution/ TMB Substrate Solution	2 vials
Stop Solution	2 N sulfuric acid	1 vial
Plate Sealers	Adhesive strip	3 strips

B. STORAGE

<table>
<tr><td>Unopened Kit</td><td colspan="2">Store at 2-8°C. Do not use past kit expiration date.</td></tr>
<tr><td rowspan="8">Opened/ Reconstituted Reagents</td><td>Wash Buffer (1×)</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>Conjugate</td></tr>
<tr><td>TMB Substrate</td></tr>
<tr><td>Assay Diluent RD1W</td></tr>
<tr><td>Calibrator Diluent RD5-3</td></tr>
<tr><td>Standard</td><td>Use a new standard for each assay. Discard after use.</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.
- Horizontal orbital microplate shaker capable of maintaining a speed of 500 ± 50 rpm.

D. PRECAUTION

- IL-6 is detectable in saliva. Take precautionary measures to prevent contamination of kit reagents while running this assay.
- 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 protective gloves, clothing, eye, and face protection. Wash hands thoroughly after handling.

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 RD5-3.

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 RD5-3.

Plasma - Collect plasma using EDTA or heparin as an anticoagulant. Centrifuge for 15 minutes at 1000 × 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 RD5-3.

Note: *Citrate plasma has not been validated for this assay.*

Urine - Aseptically collect the first urine of the day (mid-stream), voided directly into a sterile container. Centrifuge to remove particulate matter, and assay immediately or aliquot and store at ≤ -20°C. Avoid repeated freeze-thaw cycles. Samples may require dilution with Calibrator Diluent RD5-3.

B. SAMPLE PREPARATION

Cell culture supernates may require dilution due to high endogenous levels. Multiple dilutions are recommended for unknown samples.

C. REAGENT PREPARATION

Bring all reagents to room temperature before use.

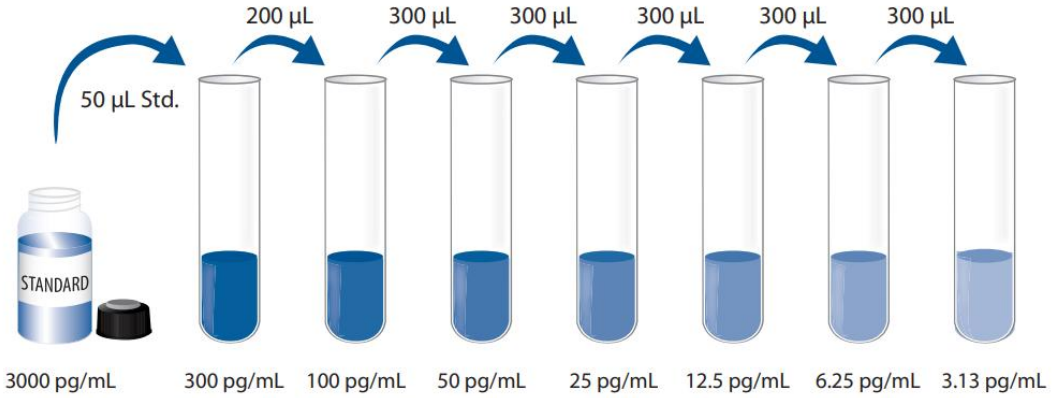
Note: *High concentrations of IL-6 are found in saliva. The use of a face mask and gloves is recommended to protect kit reagents from contamination.*

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×).

Human IL-6 Standard - Refer to the vial label for reconstitution volume. This reconstitution produces a stock solution of 3000 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 450 µL of Calibrator Diluent RD5-3 into the 300 pg/mL tube. Pipette 400 µL of Calibrator Diluent RD5-3 into the 100 pg/mL tube and 300 µL into remaining tubes. Use the stock solution to produce a dilution series (below). Mix each tube thoroughly before the next transfer. The 300 pg/mL standard serves as the high standard. Calibrator Diluent RD5-3 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

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

Note: *High concentrations of IL-6 are found in saliva. The use of a face mask and gloves is recommended to protect kit reagents from contamination.*

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 Assay Diluent RD1W to each well.
4. Add 100 µL of standard and sample per well. 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 200 µL of human IL-6 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 200 µL of TMB Substrate to each well. **Incubate for 30 minutes at room temperature. Protect from light.**
9. Add 50 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 human 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.

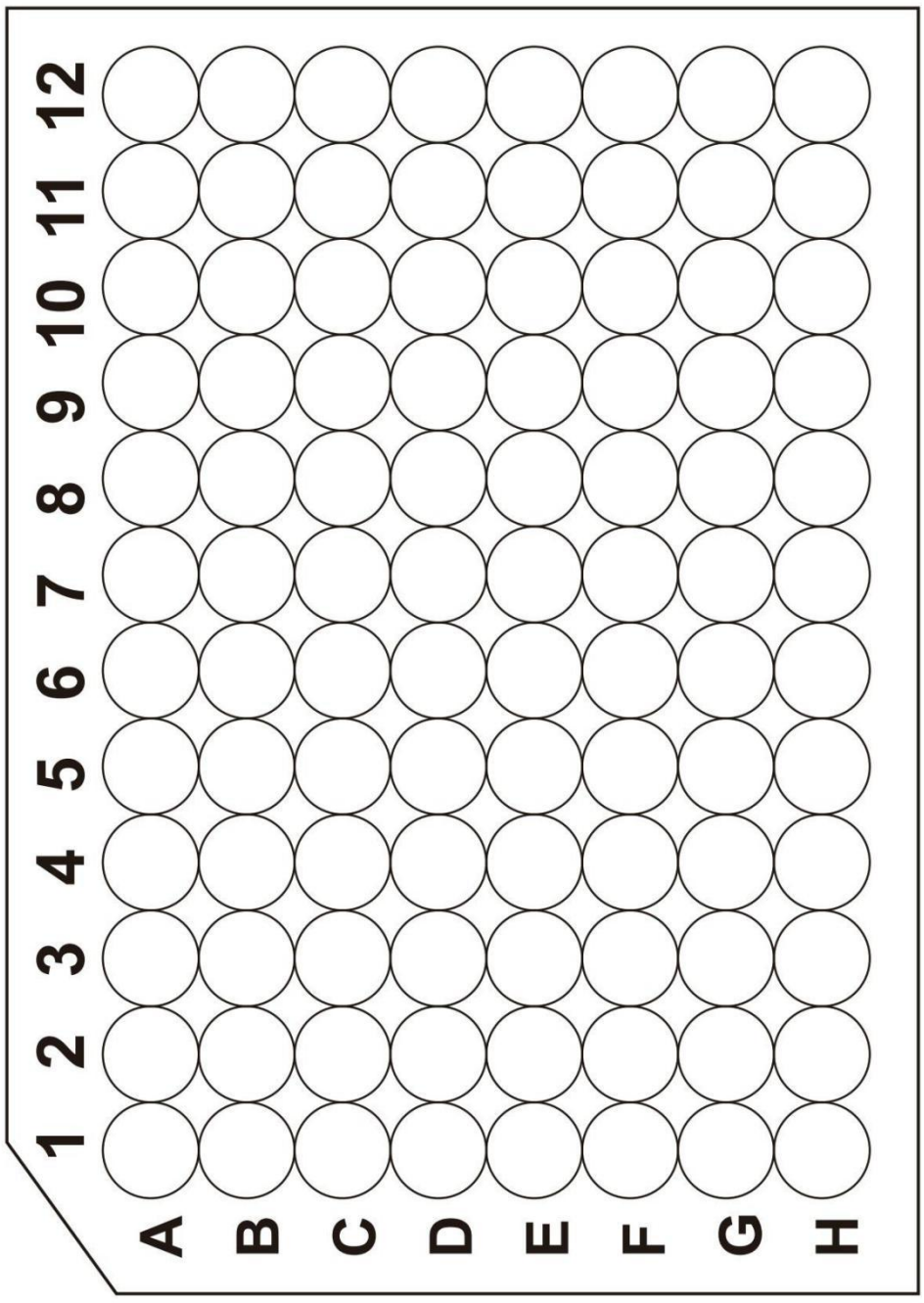
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PLATE LAYOUT

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：VAL102C

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

Bio-Techne China Co. Ltd

P: +86 (21) 52380373 P: 8009881270 F: +86 (21) 52381001

info.cn@bio-techne.com

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版本号 202604.2

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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39%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、B1B细胞和胰岛β细胞（2,11-33）。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1 pg/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-6α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抗体包被于微孔板上，样品和标准品中的人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抗体，与结合在微孔板上的人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（显色剂）。溶液颜色与结合的目标蛋白成正比；加入终止液；用酶标仪测定吸光度。

B. 检测局限

- **仅供科研使用，不可用于体外诊断；**
- 该试剂盒适用于细胞培养上清、人血清、人血浆和人尿液样本；
- 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，应将样本用标准品稀释液RD5-3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)	38.8	94.7	205	31.8	97.4	191
标准差	0.687	2.02	4.69	1.35	3.65	8.25
CV%	1.8	2.1	2.3	4.2	3.8	4.3

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)	102	82 - 122
人血清 (n=4)	96	89 - 103
人EDTA血浆 (n=4)	97	90 - 102
人肝素血浆 (n=4)	96	90 - 99
人尿液 (n=4)	97	89 - 101

C. 灵敏度

通过 24 次检测分析，人 IL-6 的最低可测剂量（MDD）范围为 0.120-0.626 pg/mL。平均 MDD 为 0.368 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 试剂盒是针对 R&D Systems 生产的高纯度重组人 IL-6 进行校准的。

NIBSC/WHO IL-6 国际标准品 89/548 (IL-6, 人 rDNA 来源) 作为效价标准，在本试剂盒中进行了评估。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) = 0.1006 × Valukine Human IL-6 value (pg/mL)

此外，第二代 NIBSC/WHO IL-6 国际标准品 21/308 (IL-6, 人重组) 作为效价标准，在本试剂盒中进行了评估。国际标准品(21/308)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 21/308 的近似单位，请使用以下公式：

NIBSC (21/308) approximate value (IU/mL) = 0.0978 × Valukine Human IL-6 value (pg/mL)

基于 2023 年 4 月的数据。

E. 线性

在不同类型样本中含有或掺入高浓度的人 IL-6，然后用标准品稀释液 RD5-3 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=3)	人血清 (n=4)	人EDTA血浆 (n=4)	人肝素血浆 (n=4)	人尿液 (n=4)
1:2	平均值/期待值（%）	97	104	103	105	100
	范围 (%)	81-109	94-112	95-111	98-111	98-105
1:4	平均值/期待值（%）	100	106	105	106	101
	范围 (%)	88-116	97-115	95-115	98-115	98-104
1:8	平均值/期待值（%）	107	108	107	110	100
	范围 (%)	95-121	99-118	99-115	102-120	98-103
1:16	平均值/期待值（%）	116	109	108	109	101
	范围 (%)	99-125	103-119	101-116	99-117	98-105

F. 样本预值

人血清/血浆样本 - 用本试剂盒检测来自明显健康的志愿者的人IL-6。本研究未获得所用样本捐献者的既往病史信息。

样本类型	可测样本平均值 (pg/mL)	可测占比 (%)	范围 (pg/mL)
人血清 (n=30)	6.90	40	ND-13.9
人EDTA血浆 (n=30)	6.82	40	ND-13.4
人肝素血浆 (n=30)	6.89	40	ND-12.9

ND=不可检测

细胞上清样本 - 人的外周血单核细胞（1×10^6 cells/mL）在RPMI中培养，RPMI中添加10%胎牛血清、50 µM β-巯基乙醇、2 mM L-谷氨酰胺、100 units/mL青霉素、100 µg/mL硫酸链霉素。PBMC 未经处理或用10 µg/mL PHA处理，培养5天。取细胞培养上清液测定IL-6含量。

条件	5天 (pg/mL)
未刺激	24.0
刺激	10,986

人尿液样本 - 采用本检测方法对来自表观健康志愿者的十份尿液样本进行了人IL-6检测。本研究未获得所用样本捐献者的既往病史信息。所有样本的检测结果均低于人IL-6的最低标准品浓度，即3.13 pg/mL。

G. 特异性

检测方法可识别天然和重组人IL-6。

将以下因子以3 ng/mL的浓度配制于标准品稀释液RD5-3中，并进行交叉反应性检测。将以下因子以3 ng/mL的浓度掺入中浓度重组人IL-6质控品中，并进行干扰性检测。未观察到以下因子存在显著的交叉反应性或干扰。

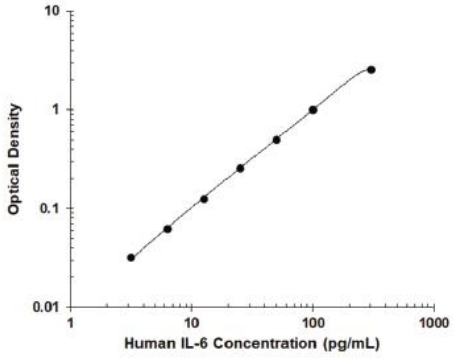
Recombinant human:	**Other recombinants:**
gp130	mouse IL-6
IL-6 Rα /gp130	rat IL-6
IL-6 sR	porcine IL-6
IL-6 R	

重组食蟹猴IL-6的交叉反应约为79%，>50 pg/mL时会干扰。

IV. 实验

标准曲线实例

该标准曲线数据仅供参考，每次实验应绘制其对应的标准曲线。



(pg/mL)	O.D.	Average	Corrected
0	0.005 0.005	0.005	—
3.13	0.032 0.032	0.032	0.027
6.25	0.063 0.063	0.063	0.058
12.5	0.124 0.125	0.125	0.120
25	0.255 0.263	0.259	0.254
50	0.504 0.504	0.504	0.499
100	0.987 1.010	0.999	0.994
300	2.569 2.575	2.572	2.567

V. 试剂盒组成及储存

A. 试剂盒组成

组成	描述	规格
Human IL-6 Microplate	包被抗人IL-6的抗体的96孔聚苯乙烯板，8孔×12条	1块板
Human IL-6 Conjugate	酶标抗人IL-6检测抗体	1瓶
Human IL-6 Standard	重组人IL-6标准品（冻干粉），参考瓶标签进行重溶	2瓶
Assay Diluent RD1W	检测液RD1W	1瓶
Calibrator Diluent RD5-3	标准品稀释液RD5-3，用于稀释标准品和样本	1瓶
Wash Buffer Concentrate（25×）	浓缩洗涤缓冲液（25×）	1瓶
TMB Substrate	TMB ELISA底物溶液/TMB底物溶液	2瓶
Stop Solution	终止液	1瓶
Plate Sealers	封板膜	3张

B. 试剂盒储存

<table>
<tr><td>未开封试剂盒</td><td colspan="2">2-8℃储存；请在试剂盒有效期内使用</td></tr>
<tr><td rowspan="7">已打开，稀释或重溶的试剂</td><td>洗涤液（1×）</td><td rowspan="6">2-8℃储存，最多30天*</td></tr>
<tr><td>终止液</td></tr>
<tr><td>酶标检测抗体</td></tr>
<tr><td>TMB底物溶液</td></tr>
<tr><td>检测液RD1W</td></tr>
<tr><td>标准品稀释液RD5-3</td></tr>
<tr><td>标准品</td><td>每次检测使用新配制的标准品，使用后丢弃</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
- 速度可调至 500 ± 50 rpm 的振荡器

D. 注意事项

- IL-6 在唾液中可被检测到。在运行本检测时，请采取预防措施以防止试剂盒组分受到污染。
- 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。使用后请彻底洗手。

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RD5-3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RD5-3稀释。

血浆样本：使用EDTA或肝素钠作为抗凝剂收集血浆。然后1000 × 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RD5-3稀释。

注意：*枸橼酸钠血浆样本尚未在本试剂盒中验证。*

尿液样本：无菌收集清晨第一次排尿的中段尿，直接排入无菌容器中。离心去除颗粒物，然后立即检测，或分装后于≤ -20°C条件下保存。避免反复冻融。样本可能需用标准品稀释液RD5-3稀释。

B. 样本准备工作

细胞培养上清可能因内源性水平较高而需要稀释。对于未知样本，建议进行多重稀释。

C. 检测前准备工作

使用前请将所有试剂放置于室温。

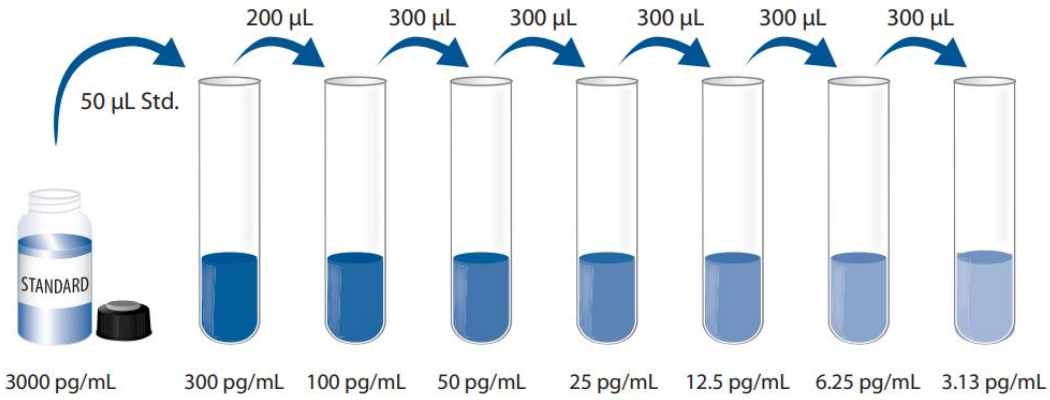
注意：*唾液中含有高浓度的IL-6。建议使用口罩和手套以保护试剂盒不受污染。*

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

人IL-6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3000 pg/mL标准品母液。轻轻震摇至少15分钟，其充分溶解。

*如有疑问，请咨询我们的技术支持。

在300 pg/mL稀释管中加入450 µL标准品稀释液RD5-3。用移液器将400 µL标准品稀释液RD5-3移到100 pg/mL试管中，并将300 µL标准品稀释液RD5-3移到其余各试管中。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。300 pg/mL标准品用作标准曲线最高点，标准品稀释液RD5-3可用作标准曲线零点（0 pg/mL）。



D. 技术小提示

- 当混合或重溶蛋白液时，尽量避免起沫；
- 为了避免交叉污染，配制不同浓度标准品、上样、加不同试剂都需要更换枪头。另外不同试剂请分别使用不同的移液槽；
- 建议15分钟内完成一块板的上样；
- 每次孵育时，正确使用封板膜可保证结果的准确性；
- TMB底物溶液在上板前应为无色，请避光保存；加入微孔板后，TMB底物将由无色变成不同深度的蓝色；
- 终止液上板顺序应同TMB底物溶液上板顺序一致；加入终止液后，孔内颜色由蓝变黄；若孔内有绿色，则表明孔内液体未混匀，请充分混合。

VII. 操作步骤

使用前请将所有试剂和样本放置于室温，建议所有的实验样本和标准品做复孔检测。

注意：*唾液中含有高浓度的IL-6。建议使用口罩和手套以保护试剂盒不受污染。*

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. 向每个孔中加入 100 µL 的检测液 RD1W。
4. 分别将不同浓度标准品和实验样本加入相应孔中，每孔100 µL。用封板膜封住反应孔，**室温500 ± 50 rpm水平振荡孵育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200 µL酶标检测抗体。用封板膜封住反应孔，**室温500 ± 50 rpm水平振荡孵育2小时**；
7. 重复第5步洗板操作；
8. 在每个微孔内加入200 µL TMB底物溶液，**室温孵育30分钟。注意避光；**
9. 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；
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。数据可以通过绘制人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

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