PRODUCT INFORMATION & MANUAL

Human IL-1β/IL-1F2 Valukine™ ELISA

Catalog Number: VAL101

For the quantitative determination of natural and recombinant human Interleukin (IL)-1β/IL-1F2 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 202604.7

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I. BACKGROUND

The Interleukin-1 (IL-1) family of proteins consists of the classic members IL-1α, IL-1β, and IL-1ra, plus IL-18, IL-33 and IL-1F5-F10. IL-1α and IL-1β bind to the same cell surface receptors and share biological functions (1). IL-1 is not produced by unstimulated cells of healthy individuals with the exception of skin keratinocytes, some epithelial cells, and certain cells of the central nervous system. However, in response to inflammatory agents, infections, or microbial endotoxins, a dramatic increase in the production of IL-1 by macrophages and various other cell types is seen. IL-1β plays a central role in immune and inflammatory responses, bone remodeling, fever, carbohydrate metabolism, and GH/IGF-I physiology. Inappropriate or prolonged production of IL-1 has been implicated in a variety of pathological conditions including sepsis, rheumatoid arthritis, inflammatory bowel disease, acute and chronic myelogenous leukemia, insulin-dependent diabetes mellitus, atherosclerosis, neuronal injury, and aging-related diseases (2-5).

IL-1α and IL-1β are structurally related polypeptides that show approximately 25% homology at the amino acid level. Both are synthesized as 31 kDa precursors that are subsequently cleaved into mature proteins of approximately 17.5 kDa (6, 7). Cleavage of the IL-1βprecursor by Caspase-1/ICE is a key step in the inflammatory response (2, 8). Neither IL-1α nor IL-1βcontains a typical hydrophobic signal peptide (9-11), but evidence suggests that these factors can be secreted by non-classical pathways (12, 13). A portion of unprocessed IL-1α can be presented on the cell membrane and may retain biological activity (14). The precursor form of IL-1β, unlike the IL-1α precursor, shows little or no biological activity in comparison to the processed form (13, 15). Both unprocessed and mature forms of IL-1β are exported from the cell.

IL-1α and IL-1β exert their effects through immunoglobulin superfamily receptors that additionally bind IL-1ra. The 80 kDa transmembrane type I receptor (IL-1 RI) is expressed on T cells, fibroblasts, keratinocytes, endothelial cells, synovial lining cells, chondrocytes, and hepatocytes (16, 17). The 68 kDa transmembrane type II receptor (IL-1 RII) is expressed on B cells, neutrophils, and bone marrow cells (18). The two IL-1 receptor types show approximately 28% homology in their extracellular domains but differ significantly in that the type II receptor has a cytoplasmic domain of only 29 amino acids (aa), whereas the type I receptor has a 213 aa cytoplasmic domain. IL-1 RII does not appear to signal in response to IL-1 and may function as a decoy receptor that attenuates IL-1 function (19). The IL-1 receptor accessory protein (IL-1 RAcP) associates with IL-1 RI and is required for IL-1 RI signal transduction (20). IL-1ra is a secreted molecule that functions as a competitive inhibitor of IL-1 (21, 22). Soluble forms of both IL-1 RI and IL-1 RII have been detected in human plasma, synovial fluids, and the conditioned media of several human cell lines (23, 24). In addition, IL-1 binding proteins that resemble soluble IL-1 RII are encoded by vaccinia and cowpox viruses (25).

II. OVERVIEW

A. PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. An antibody specific for human IL-1β has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any human IL-1β present is bound by the immobilized antibody. After washing away any unbound substances, an enzyme-linked antibody specific for human IL-1β 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 IL-1β 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 and human 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 RD5-5/RD6C 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.

CELL CULTURE SUPERNATE ASSAY

	Intra-assay Precision				**Inter-assay Precision**		
Sample	1	2	3	4	1	2	3
Mean (pg/mL)	12.6	30.4	69.2	192	30.8	120	194
Standard Deviation	0.3	1.0	1.6	6.5	2.2	4.1	7.9
CV%	2.4	3.3	2.3	3.4	7.1	3.4	4.1

SERUM/PLASMA ASSAY

	Intra-assay Precision				**Inter-assay Precision**		
Sample	1	2	3	4	1	2	3
Mean (pg/mL)	18.9	30.2	122	181	29.8	118	193
Standard Deviation	1.6	1.0	5.4	5.0	2.5	5.0	8.0
CV%	8.5	3.3	4.4	2.8	8.4	4.2	4.1

B. RECOVERY

The recovery of human IL-1β spiked to different levels throughout the range of the assay in various matrices was evaluated.

Sample Type	**Average % Recovery**	**Range%**
Cell culture media	97	80-111
Human serum	95	87-110
Human EDTA plasma	86	81-100
Human heparin plasma	82	76-100
Human citrate plasma	93	83-110

C. SENSITIVITY

The minimum detectable dose (MDD) of IL-1β is typically less than 1.0 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-1β. The non-WHO reference material for IL-1β 86/552 was evaluated in this kit. The dose response curve of the reference material parallels the Valukine standard curve. To convert sample values obtained with the Valukine kit to approximate NIBSC (86/552) units, use the equation below.

NIBSC (86/552) approximate value (U/mL) = 0.098 × Valukine Human IL-1β value (pg/mL)

E. LINEARITY

To assess the linearity of the assay, samples were containing or spiked with high concentrations of human IL-1β in various matrices and diluted with Calibrator Diluent RD5-5/RD6C to produce samples with values within the dynamic range of the assay.

Dilution		Cell culture media (n=3)	Human serum (n=4)	Human EDTA plasma (n=4)	Human heparin plasma (n=4)	Human citrate plasma (n=4)
1:2	Average % of Expected	101	99	99	99	101
	Range (%)	/	98-99	95-101	95-102	96-109
1:4	Average % of Expected	103	97	99	100	99
	Range (%)	103-104	94-100	93-102	96-102	87-104
1:8	Average % of Expected	106	95	100	94	91
	Range (%)	/	93-98	95-106	88-99	87-99
1:16	Average % of Expected	108	89	91	91	87
	Range (%)	107-109	87-91	86-96	84-96	81-93

F. SAMPLE VALUES

Cell Culture Supernates - Human peripheral blood mononuclear cells (1×10^6 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. Cells were stimulated with the agents listed in the table below. Aliquots of the cell culture supernate were removed on days 1, 3, and 5 and assayed for levels of human IL-1β.

Stimulant	Day 1 (pg/mL)	Day 3 (pg/mL)	Day 5 (pg/mL)
10 µg/mL PHA	2185	2004	2383
10 µg/mL PHA + 10 ng/mL rhIL-2	1938	1973	2839
50 ng/mL PMA	1767	1027	1159
50 ng/mL LPS	4158	2145	1308

Human serum/plasma - Forty serum and plasma samples from apparently healthy volunteers were evaluated for the presence of human IL-1β in this assay. No medical histories were available for the donors used in this study. All samples measured less than the lowest IL-1β standard, 3.9 pg/mL.

G. SPECIFICITY

This assay recognizes both natural and recombinant human IL-1β. This assay also recognizes recombinant rhesus macaque IL-1β.

The factors listed below were prepared at 50 ng/mL in calibrator diluent and assayed for cross-reactivity. Preparations of the following factors at 100 ng/mL in a mid-range recombinant human IL-1β control were assayed for interference. No significant cross-reactivity or interference was observed with the following.

Recombinant human	**Recombinant mouse**	**Recombinant equine**
IL-1α	IL-1α	IL-1α
IL-1F7	IL-1β	IL-1ra
IL-1ra	IL-33	**Other recombinants**
IL-1 RAcP	SIGIRR	cotton rat IL-1α
IL-1 RAPL1	ST2	canine IL-1β
IL-1 RAPL2	**Recombinant rat**	guinea pig IL-1β
IL-1 Rrp2	IL-1α	
IL-18	IL-1ra	
IL-18 Rα	**Recombinant porcine**	
IL-18 Rβ	IL-1α	
IL-33	IL-1β	
IL-36α	IL-1ra	
IL-36β		
IL-36γ		
IL-36ra		
IL-38		
SIGIRR		
ST2		

Recombinant human Pro-IL-1β does not interfere but does cross-react approximately 6.1% in this assay.

Recombinant rat IL-1β does not interfere but does cross-react approximately 0.2% in this assay.

Recombinant cotton rat IL-1β does not interfere but does cross-react approximately 0.4% in this assay.

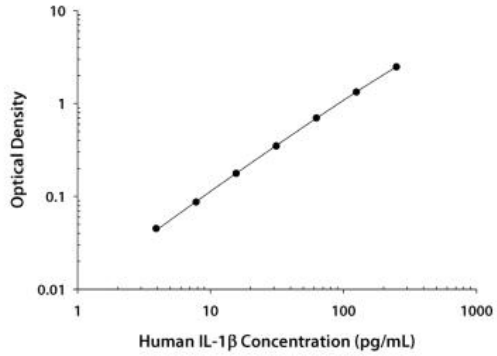
Recombinant human IL-1 RI and IL-1 RII do not cross-react but do interfere at concentrations > 10,000 pg/mL.

IV. EXPERIMENT

EXAMPLE STANDARD

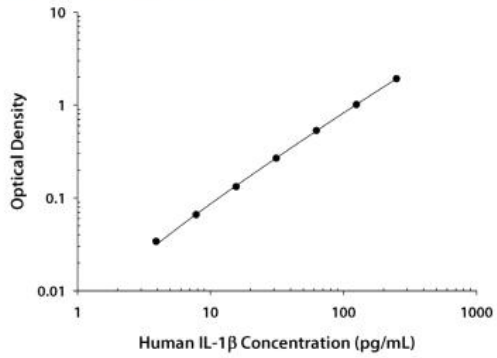
These standard curves are provided for demonstration only. A standard curve should be generated for each set of samples assayed.

CELL CULTURE SUPERNATE ASSAY



(pg/mL)	O.D.	Average	Corrected
0	0.023 0.027	0.025	—
3.9	0.070 0.071	0.070	0.045
7.8	0.109 0.115	0.112	0.087
15.6	0.197 0.206	0.202	0.177
31.3	0.373 0.374	0.374	0.349
62.5	0.715 0.733	0.724	0.699
125	1.349 1.366	1.358	1.333
250	2.495 2.517	2.506	2.481

SERUM/PLASMA ASSAY



(pg/mL)	O.D.	Average	Corrected
0	0.022 0.026	0.024	—
3.9	0.057 0.058	0.058	0.034
7.8	0.088 0.091	0.090	0.066
15.6	0.156 0.157	0.156	0.132
31.3	0.290 0.292	0.291	0.267
62.5	0.549 0.563	0.556	0.532
125	1.034 1.036	1.035	1.011
250	1.933 1.942	1.938	1.914

V. KIT COMPONENTS AND STORAGE

A. MATERIALS PROVIDED

Parts	Description	Size
Human IL-1β Microplate	96 well polystyrene microplate (12 strips of 8 wells) coated with an antibody against human IL-1β	1 plate
Human IL-1β Conjugate	Antibody against human IL-1β conjugated to horseradish peroxidase	1 vial
Human IL-1β Standard	Recombinant human IL-1β in a buffered protein base; lyophilized. Refer to the vial label for reconstitution volume.	1 vial
Assay Diluent RD1-83	A buffered protein base with preservatives. *May contain a precipitate. Mix well before and during use. For human serum/plasma samples.*	1 vial
Calibrator Diluent RD5-5	A buffered protein base used to dilute standard and samples. *For cell culture supernate samples.*	1 vial
Calibrator Diluent RD6C	A buffered protein base used to dilute standard and samples. *For human serum/plasma 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 RD1-83</td></tr>
<tr><td>Calibrator Diluent RD5-5</td></tr>
<tr><td>Calibrator Diluent RD6C</td></tr>
<tr><td>Standard</td><td>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>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

- IL-1β is detectable in saliva and sweat. Take precautionary measures to prevent contamination of the kit reagents while running the assay.
- Calibrator Diluent RD6C contains sodium azide which may react with lead and copper plumbing to form explosive metallic azides. Flush with large volumes of water during disposal.
- The Stop Solution provided with this kit is an acid solution.
- Some components in this kit contain a preservative which may cause an allergic skin reaction. Avoid breathing mist.
- 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 RD5-5.

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 RD6C.

Plasma - Collect plasma using EDTA, heparin, or citrate 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 RD6C.

B. REAGENT PREPARATION

Bring all reagents to room temperature before use.

Note: *High concentrations of IL-1β are found in saliva and sweat. Wear a face mask and gloves be used to protect kit reagents from contamination.*

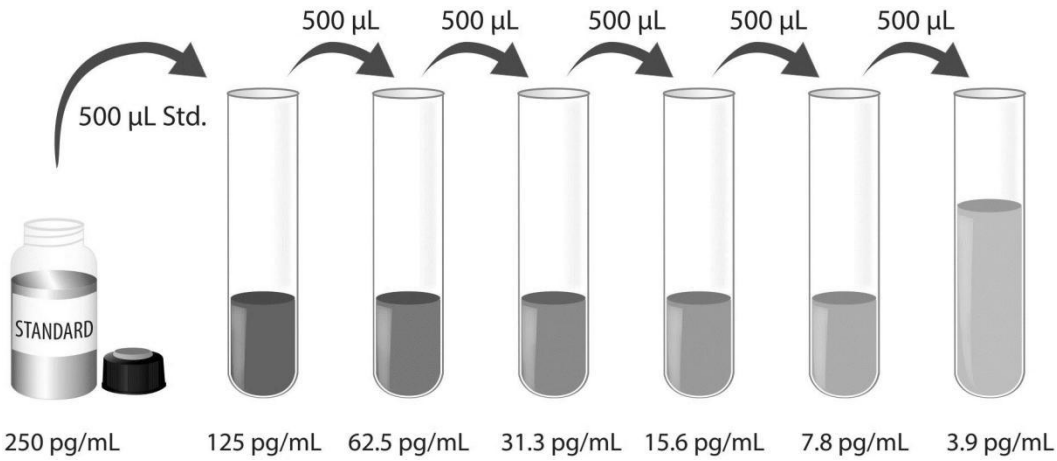
Wash Buffer (1×) - If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. Dilute 20 mL of Wash Buffer Concentrate (25×) into deionized or distilled water to prepare 500 mL of Wash Buffer (1×).

Human IL-1β Standard - Refer to the vial label for the reconstitution volume*. Reconstitute the Human IL-1β Standard with Calibrator Diluent RD5-5 (*for cell culture supernate samples*) or Calibrator Diluent RD6C (*for human serum/plasma samples*). This reconstitution produces a stock solution of 250 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 500 µL of Calibrator Diluent RD5-5 (*for cell culture supernate samples*) or Calibrator Diluent RD6C (*for human serum/plasma samples*) into each tube. Use the stock solution to produce a dilution series (below). Mix each tube thoroughly before the

next transfer. The undiluted Human IL-1β Standard (250 pg/mL) serves as the high standard. The appropriate calibrator diluent serves as the zero standard (0 pg/mL).



C. 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: *IL-1β is detectable in saliva and sweat. Take precautionary measures to prevent contamination of the kit reagents while running the assay.*

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. **For Cell Culture Supernate Samples:** Proceed to step 4.

   For Human Serum/Plasma Samples: Add 50 µL of Assay Diluent RD1-83 to each well. *Assay Diluent RD1-83 may contain a precipitate. Mix well before and during use.*
4. Add 200 µL of standard and 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.
5. Aspirate each well and wash, repeating the process twice times for a total of three 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-1β Conjugate to each well. Cover with a new adhesive strip.

   For Cell Culture Supernate Samples: **Incubate for 1 hour at room temperature.**
   For **Human** Serum/Plasma Samples: **Incubate for 2 hours at room temperature.**
7. Repeat the aspiration/wash as in step 5.
8. Add 200 µL of TMB Substrate to each well. **Incubate for 20 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-1β 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.

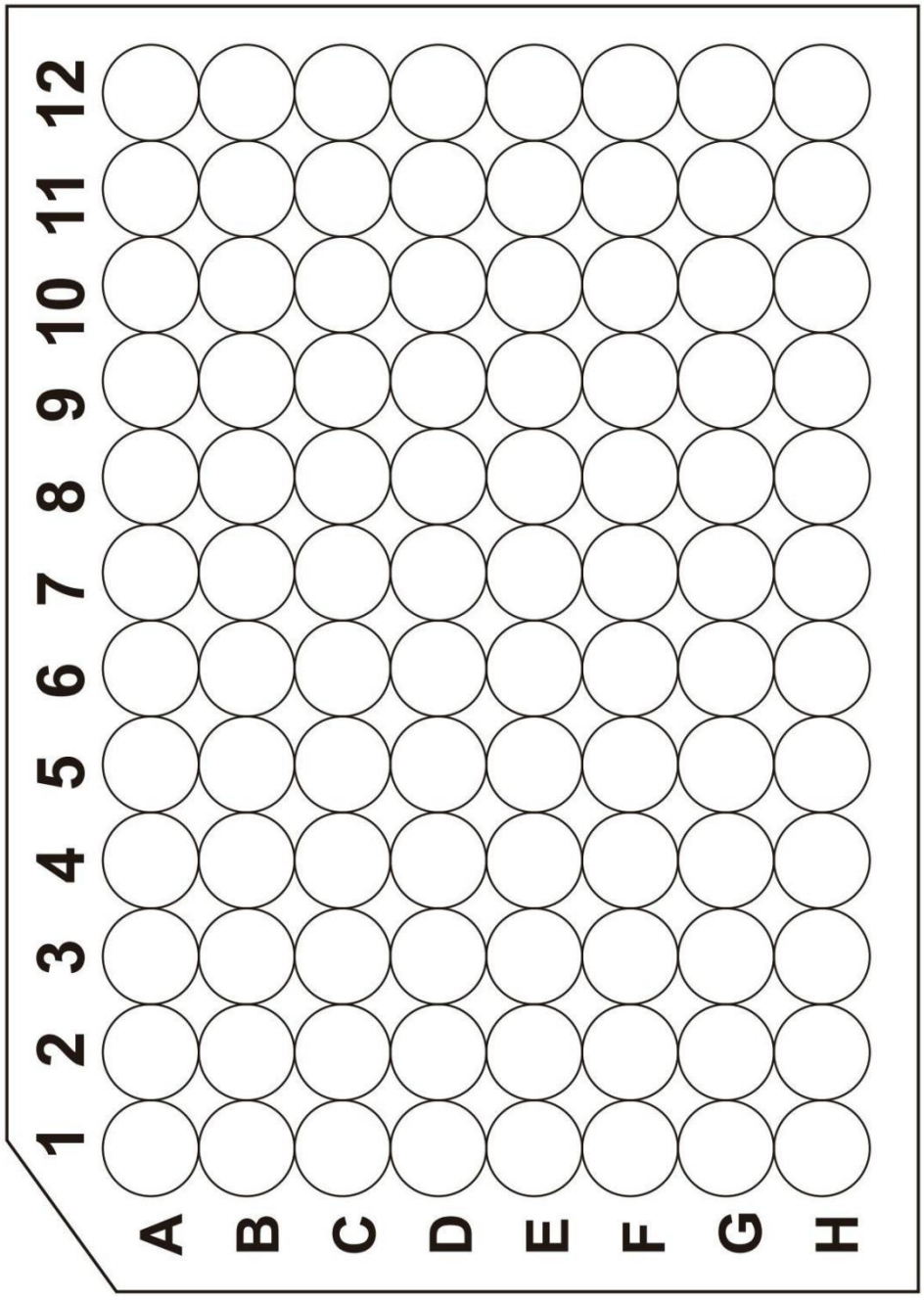
If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.

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-1β/IL-1F2 Valukine™ ELISA 试剂盒

目录号：VAL101

适用于定量检测天然和重组人白介素 1β（IL-1β）/白介素 1F2（IL-1F2）的浓度

科研专用，不可用于临床诊断

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有效期详见试剂盒包装标签

Novus 试剂盒确保在你收货日期 3 个月内有效

版本号 202604.7

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I. 背景

白细胞介素1 (IL-1)家族蛋白包括经典成员IL-1α、IL-1β和IL-1ra，IL-18、IL-33和IL-1F5-F10。IL-1α和IL-1β有相同的细胞表面结合受体，形式相同的生物学功能（1）。健康人未刺激的细胞是不产生IL-1的，只有皮肤角质形成细胞、一些上皮细胞，以及中枢神经系统的某些细胞是例外。但在响应炎症因子、感染、或微生物内毒素时，IL-1在巨噬细胞和其他类型细胞表达急剧增加。IL-1β在免疫和炎症应答、骨重塑、发热、碳水化合物代谢、GH/IGF-I的生理过程中起重要作用。多种疾病与 IL-1表达失调或延时表达相关，其中包括败血症、类风湿关节炎、炎性肠道疾病、急性和慢性髓细胞白血病、胰岛素依赖型糖尿病、动脉粥状硬化、神经损伤和老年退化有关的疾病（2-5）。

IL-1α和IL-1β是结构相关的多肽，在氨基酸水平约有25%的同源性。两者都是先合成为 31kDa前体，然后裂解为大约17.5 kDa的成熟蛋白（6, 7）。由Caspase-1/ICE对IL-1β的前体切割是炎症反应的关键步骤（2, 8）。尽管IL-1α和IL-1β都不含有一个典型的疏水信号肽（9-11），但有证据表明，它们可以经非经典途径分泌到胞外（12, 13）。未处理的IL-1α部分可在细胞膜上展示，并可能保留生物活性（14）。与IL-1α前体不同，IL-1β前体与成熟的IL-1β相比，具有很少或根本不具有生物活性（13, 15）。IL-1β前体与成熟的IL-1β都运至胞外。

IL-1α和IL-1β通过免疫球蛋白超家族受体与IL-1ra结合发挥其效应。80 kDa的I型跨膜受体（IL-1RI）在多种细胞中表达，包括T细胞、成纤维细胞、角质形成细胞、内皮细胞、滑膜衬里细胞、软骨细胞和肝细胞（16, 17）。68 kDa的II型跨膜受体（IL-1 RII）在B细胞、中性粒细胞和骨髓细胞中表达（18）。IL-1RI和IL-1RII的胞外区域享有28%的同源性。但其他区域有显著不同：II型受体的胞内域只有29个氨基酸，而I型受体的胞内域有213个氨基酸。IL-1RII似乎对IL-1信号没有响应，其功能是作为诱骗受体从而削弱IL-1的作用（19）。IL-1受体配体蛋白（IL-1 RAcP）与IL-1RI相互作用是IL-1RI信号传导所必需的（20）。IL-1ra是分泌型分子，是IL-1的竞争性抑制剂（21, 22）。可溶性IL-1RI和IL-1RII存在于人的血浆、关节液、及几种细胞株的条件培养基中（23, 24）。此外，牛痘病毒编码类似于可溶性IL-1RII的IL-1结合蛋白（25）。

II. 概述

A. 检测原理

本实验采用双抗体夹心ELISA法。抗人IL-1β抗体包被于微孔板上，样品和标准品中的人IL-1β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-1β检测抗体进行孵育。洗涤去除未结合的试剂后，加入TMB底物溶液（显色剂）。溶液颜色与结合目标蛋白成正比；加入终止液；用酶标仪测定吸光度。

B. 检测局限

- **仅供科研使用，不可用于体外诊断；**
- 该试剂盒适用于细胞培养上清、人血清和人血浆样本；
- 请在试剂盒有效期内使用；
- 不同试剂盒及不同批号试剂盒的组分不能混用；
- 样本值若大于标准曲线的最高值，应将样本用标准品稀释液RD5-5/RD6C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。

细胞培养上清

	板内精确度				板间精确度		
样本	1	2	3	4	1	2	3
平均值 (pg/mL)	12.6	30.4	69.2	192	30.8	120	194
标准差	0.3	1.0	1.6	6.5	2.2	4.1	7.9
CV%	2.4	3.3	2.3	3.4	7.1	3.4	4.1

血清/血浆

	板内精确度				板间精确度		
样本	1	2	3	4	1	2	3
平均值 (pg/mL)	18.9	30.2	122	181	29.8	118	193
标准差	1.6	1.0	5.4	5.0	2.5	5.0	8.0
CV%	8.5	3.3	4.4	2.8	8.4	4.2	4.1

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-1β，测定其回收率。

样本类型	平均回收率（%）	范围（%）
细胞培养基	97	80-111
人血清	95	87-110
人EDTA血浆	86	81-100
人肝素血浆	82	76-100
人枸橼酸钠血浆	93	83-110

C. 灵敏度

人IL-1β的最低可测剂量（MDD）一般小于1.0 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

本免疫检测方法以高度纯化的重组人IL-1β为标准进行校准。非WHO来源的IL-1β参考物质（86/552）已在本试剂盒中经过评估。该参考物质的剂量反应曲线与Valukine标准曲线相平行。如需将使用Valukine试剂盒获得的样本数值换算为近似的NIBSC（86/552）单位，请使用以下公式：

NIBSC（86/552）近似值（U/mL）= 0.098 × Valukine人IL-1β检测值（pg/mL）

E. 线性

在不同类型样本中含有或者掺入高浓度的人IL-1β，然后用标准品稀释液RD5-5/RD6C将样本稀释到检测范围内，测定其线性。

稀释倍数		细胞培养基(n=3)	人血清(n=4)	人EDTA血浆(n=4)	人肝素血浆(n=4)	人枸橼酸钠血浆(n=4)
1:2	平均值/期待值（%）	101	99	99	99	101
	范围（%）	/	98-99	95-101	95-102	96-109
1:4	平均值/期待值（%）	103	97	99	100	99
	范围（%）	103-104	94-100	93-102	96-102	87-104
1:8	平均值/期待值（%）	106	95	100	94	91
	范围（%）	/	93-98	95-106	88-99	87-99
1:16	平均值/期待值（%）	108	89	91	91	87
	范围（%）	107-109	87-91	86-96	84-96	81-93

F. 样本预值

细胞培养上清样本 - 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RPMI培养基中，细胞培养基还含有2 mM L-谷氨酰胺、50 μM β-巯基乙醇、100 U/mL青霉素，100 μg/mL硫酸链霉素。用下表中列出的试剂刺激细胞。分别在第1天、第3天和第5天取细胞培养上清液的等分试样，检测其中人IL-1β的水平。

刺激剂	第一天(pg/mL)	第三天(pg/mL)	第五天(pg/mL)
10 μg/mL PHA	2185	2004	2383
10 μg/mL PHA + 10 ng/mL rhIL-2	1938	1973	2839
50 ng/mL PMA	1767	1027	1159
50 ng/mL LPS	4158	2145	1308

人血清/血浆样本 - 本试剂盒评估了来自表面健康志愿者的40份人血清和人血浆样本中是否存在人IL-1β。本研究所用样本捐赠者的既往病史不详。所有样本的检测结果均低于人IL-1β的最低标准品浓度，即3.9 pg/mL。

G. 特异性

本检测方法可识别天然和重组人IL-1β。本检测方法也可识别重组恒河猴IL-1β。

将以下因子以50 ng/mL的浓度配制于标准品稀释液中，并进行交叉反应性检测。将以下因子以100 ng/mL的浓度配制于中浓度重组人IL-1β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	**Recombinant mouse**	**Recombinant equine**
IL-1α	IL-1α	IL-1α
IL-1F7	IL-1β	IL-1ra
IL-1ra	IL-33	**Other recombinants**
IL-1 RAcP	SIGIRR	cotton rat IL-1α
IL-1 RAPL1	ST2	canine IL-1β
IL-1 RAPL2	**Recombinant rat**	guinea pig IL-1β
IL-1 Rrp2	IL-1α	
IL-18	IL-1ra	
IL-18 Rα	**Recombinant porcine**	
IL-18 Rβ	IL-1α	
IL-33	IL-1β	
IL-36α	IL-1ra	
IL-36β		
IL-36γ		
IL-36ra		
IL-38		
SIGIRR		
ST2		

重组人Pro-IL-1β在本检测中不产生干扰，但存在约6.1%的交叉反应性。

重组大鼠IL-1β在本检测中不产生干扰，但存在约0.2%的交叉反应性。

重组棉鼠IL-1β在本检测中不产生干扰，但存在约0.4%的交叉反应性。

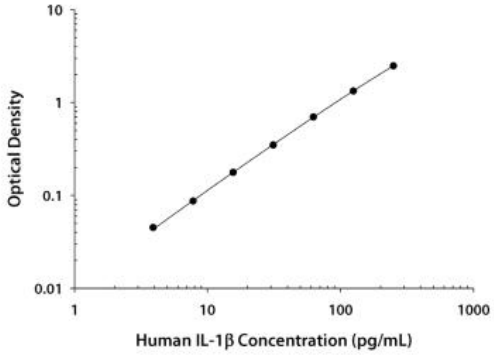
重组人IL-1 RI和IL-1 RII不产生交叉反应，但在浓度高于10,000 pg/mL时存在干扰。

IV. 实验

标准曲线实例

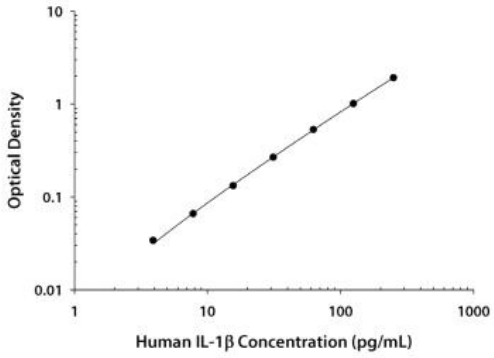
该标准曲线数据仅供参考，每次实验应绘制其对应的标准曲线。

CELL CULTURE SUPERNATE ASSAY



(pg/mL)	O.D.	Average	Corrected
0	0.023 0.027	0.025	—
3.9	0.070 0.071	0.070	0.045
7.8	0.109 0.115	0.112	0.087
15.6	0.197 0.206	0.202	0.177
31.3	0.373 0.374	0.374	0.349
62.5	0.715 0.733	0.724	0.699
125	1.349 1.366	1.358	1.333
250	2.495 2.517	2.506	2.481

SERUM/PLASMA ASSAY



(pg/mL)	O.D.	Average	Corrected
0	0.022 0.026	0.024	—
3.9	0.057 0.058	0.058	0.034
7.8	0.088 0.091	0.090	0.066
15.6	0.156 0.157	0.156	0.132
31.3	0.290 0.292	0.291	0.267
62.5	0.549 0.563	0.556	0.532
125	1.034 1.036	1.035	1.011
250	1.933 1.942	1.938	1.914

V. 试剂盒组成及储存

A. 试剂盒组成

组成	描述	规格
Human IL-1β Microplate	包被抗人IL-1β抗体的96孔聚苯乙烯板，8孔×12条	1块板
Human IL-1β Conjugate	酶标检测抗人IL-1β抗体	1瓶
Human IL-1β Standard	人IL-1β标准品（冻干），参考瓶身标签进行重溶	1瓶
Assay Diluent RD1-83	检测液RD1-83。*可能含有沉淀。使用前及使用过程中请充分混匀。适用于人血清/血浆样本。*	1瓶
Calibrator Diluent RD5-5	标准品稀释液RD5-5，*适用于细胞培养上清样本。*	1瓶
Calibrator Diluent RD6C	标准品稀释液RD6C，*适用于人血清/血浆样本。*	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="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>酶标检测抗体</td></tr>
<tr><td>TMB底物溶液</td></tr>
<tr><td>检测液RD1-83</td></tr>
<tr><td>标准品稀释液RD5-5</td></tr>
<tr><td>标准品稀释液RD6C</td><td>分装，-20℃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

- IL-1β在唾液和汗液中可被检测到。在运行检测时，请采取预防措施以防止试剂盒组分受到污染。
- 标准品稀释液RD6C含有叠氮化钠，该物质可能与铅管或铜管反应形成易爆的金属叠氮化物。废弃处置时应用大量水冲洗。
- 本试剂盒提供的终止液为酸性溶液。
- 本试剂盒中的某些组分含有防腐剂，可能引起过敏性皮肤反应。避免吸入雾状液滴。
- 穿戴防护手套、防护服、眼罩和面罩。操作后彻底清洗双手。

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RD5-5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RD6C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 × 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RD6C稀释。

B. 检测前准备工作

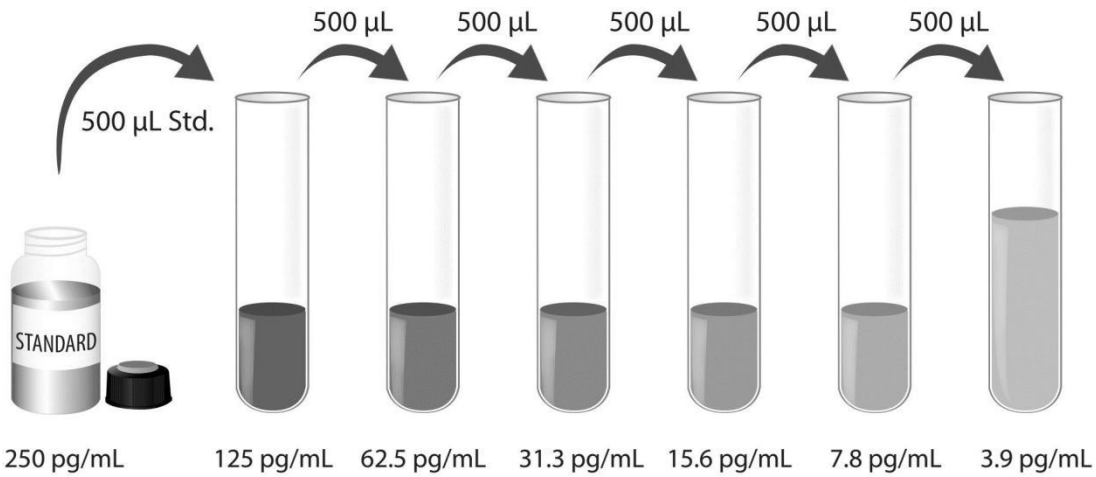
使用前请将所有试剂放置于室温。
注意： *唾液和汗液中存在高浓度的IL-1β。请佩戴口罩和手套，以保护试剂盒组分免受污染。*

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-1β标准品：冻干标准品的重溶体积请参考瓶身标签*。使用标准品稀释液RD5-5（*适用于细胞培养上清样本*）或标准品稀释液RD6C（*适用于人血清/血浆样本*）复溶人IL-1β标准品。复溶后得到浓度为250 pg/mL的标准品储备液。在进行稀释前，让标准品静置至少15分钟，并伴随温和的摇动。

*如有疑问，请咨询我们的技术支持。

向每个试管中加入500 µL的标准品稀释液RD5-5（*适用于细胞培养上清样本*）或标准品稀释液RD6C（*适用于人血清/血浆样本*）。使用储备液按下图进行系列稀释。在每次转移前，将每个试管充分混匀。未稀释的人IL-1β标准品（250 pg/mL）作为最高浓度标准点。相应的标准品稀释液作为零标准点（0 pg/mL）。



C. 技术小提示

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

VII. 操作步骤

使用前请将所有试剂和样本放置于室温，建议所有的实验样本和标准品做复孔检测。

注意： *IL-1β在唾液和汗液中可被检测到。在运行检测时，请采取预防措施以防止试剂盒组分受到污染。*

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. 对于**细胞培养上清**样本： 继续执行步骤4；

   对于人血清/血浆样本： 向每个孔中加入50 µL的检测液RD1-83。*检测液RD1-83中可能含有沉淀。使用前及使用过程中请充分混匀*；
4. 分别将不同浓度标准品和实验样本加入相应孔中，每孔200 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；
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2次，共洗3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；

   对于细胞培养上清样本： 在室温下孵育1小时；
   对于人血清/血浆样本： 在室温下孵育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20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-1β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

