PRODUCT INFORMATION & MANUAL

Human IL-1β/IL-1F2 Valukine™ ELISA

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

Version202006.3

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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-1rα, 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. A monoclonal antibody specific for IL-1β has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any IL-1β present is bound by the immobilized antibody. After washing away any unbound substances, an enzyme-linked polyclonal antibody specific for IL-1β 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-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, 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)	20.7	65.0	141	21.4	66.9	137
Standard Deviation	1.20	4.0	5.3	2.3	6.1	9.3
CV%	5.8	6.2	3.8	10.5	9.2	6.8

B. RECOVERY

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

Sample Type	Average % Recovery	Range
Cell culture media (n=4)	97	85 - 107%
Serum (n=4)	89	84 – 92%
Plasma (n=4)	94	93 - 97%

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 highly purified *E. coli*-expressed recombinant human IL-1β produced at R&D Systems.

E. LINEARITY

To assess the linearity of the assay, samples were spiked with high concentrations of human IL-1β 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=4)**	**Plasma (n=4)**
1:2	Average % of Expected	102	99	96
	Range (%)	98 - 107	98 - 101	92-100
1:4	Average % of Expected	103	102	101
	Range (%)	101 - 110	101 - 103	91-105
1:8	Average % of Expected	106	102	105
	Range (%)	103 - 110	101 - 102	103-110
1:16	Average % of Expected	107	105	104
	Range (%)	105 - 113	104 - 106	97-109

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, 50µM β-mercaptoethanol, 2 mM L-glutamine, 100 U/mL penicillin, and 100µg/mL streptomycin sulfate. Cells were stimulated as described below. Aliquots of the cell culture supernate was removed and assayed for levels of natural 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

Serum - Four serum samples were evaluated for the presence of human IL-1β in this assay. All samples measured below the lowest standard, 3.9 pg/mL.

Plasma - Four human plasma samples were evaluated for the presence of human IL-1β in this assay. All samples measured ranged from 20.0 to 47.1 pg/mL with an average of 32.3 pg/mL.

G. SPECIFICITY

This assay recognizes both natural and recombinant human IL-1β. 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-1β control were assayed for interference. No significant cross-reactivity or interference was observed.

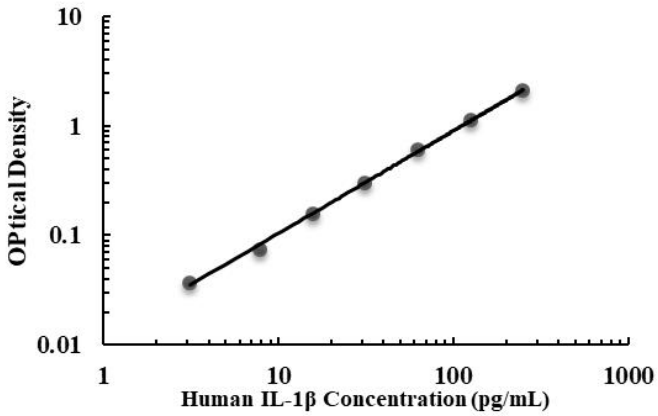
Recombinant human			**Recombinant mouse**	
EGF	IL-2	IL-9	IL-1	IL-13
G-CSF	IL-2 sRα	IL-10	IL-1	TNF-α
GM-CSF	IL-3	IL-11	IL-3	
sgp130	IL-3 sRα	IL-12	IL-4	
GRO	IL-4	IL-13	IL-5	
GRO	IL-4 sR	IL-18	IL-5 sRα	
IFN-β	IL-5	PDGF-AA	IL-6	
IGF-I	IL-5 sRβ	PDGF-AB	IL-7	
IGF-II	IL-6	PDGF-BB	IL-9	
IL-1a	IL-6sR	VEGF	IL-10	
IL-1ra	IL-7			

Recombinant human IL-1 sRI and IL-1 sRII do not cross-react in this assay. However, interference was observed at concentrations greater than 10,000 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	OD	Average	Corrected
0	0.044 0.047	0.046	-
3.1	0.081 0.084	0.083	0.037
7.8	0.120 0.121	0.121	0.075
15.6	0.197 0.212	0.205	0.159
31.3	0.346 0.350	0.348	0.303
62.5	0.655 0.656	0.656	0.610
125	1.202 1.131	1.167	1.121
250	2.097 2.155	2.126	2.081

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 a mouse monoclonal antibody against human IL-1β	1 plate
Human IL-1β Conjugate	polyclonal 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	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
Color Reagent A	stabilized hydrogen peroxide	1 vial
Color Reagent B	stabilized chromogen (tetramethylbenzidine)	1 vial
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="7">Opened/
Reconstituted
Reagents</td><td>Diluted Wash Solution</td><td rowspan="6">May be stored for up to 1 month at 2-8°C.*</td></tr>
<tr><td>Stop Solution</td></tr>
<tr><td>Diluent 1×</td></tr>
<tr><td>Conjugate</td></tr>
<tr><td>Unmixed Color Reagent A</td></tr>
<tr><td>Unmixed Color Reagent B</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

- The Stop Solution provided with this kit is an acid solution. Wear eye, hand, face, and clothing protection when using this material.
- IL-1β is detectable in saliva. Take the necessary precautions to prevent contamination of the kit reagents.

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 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 2-fold dilution. A suggested 2-fold dilution is 100 µL of sample + 100 µL of Calibrator Diluent (1×).

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.

Substrate Solution - Color Reagent A and B should be mixed together in equal volumes within 15 minutes of use. Protect from light. 200 µL of the resultant mixture is required per well.

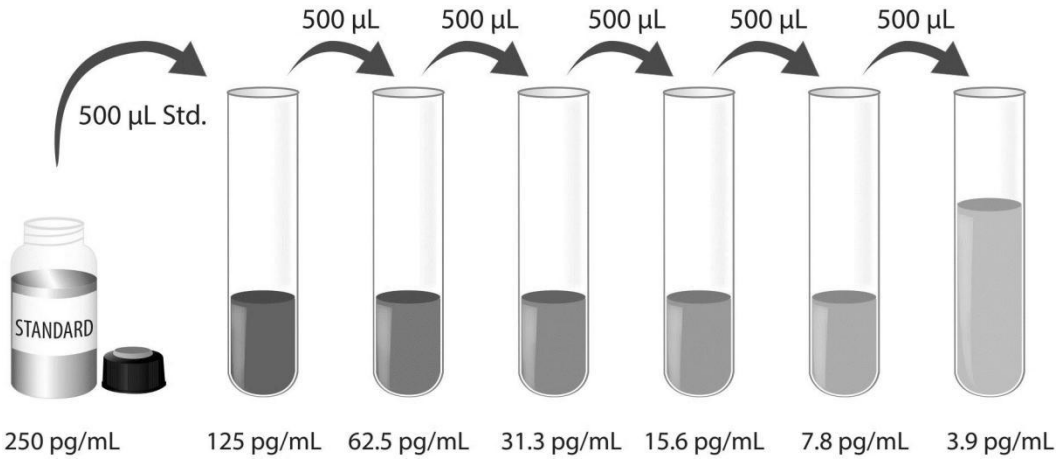
Diluent 1× - Add 20 mL of Calibrator Diluent 5× into 80 mL of deionized or distilled water to prepare 100 mL of Diluent 1×.

IL-1β Standard – Refer to the vial label for the reconstitution volume*. 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 Diluent 1× into each tube. Use the stock solution to produce a dilution series (below). Mix each tube thoroughly before the next transfer. The 250 pg/mL standard serves as the high standard. 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.
- Substrate Solution should remain colorless until added to the plate. Keep Substrate Solution protected from light. Substrate Solution should change from colorless to gradations of blue.
- Stop Solution should be added to the plate in the same order as the Substrate Solution. The color developed in the wells will turn from blue to yellow upon addition of the Stop Solution. Wells that are green in color indicate that the Stop Solution has not mixed thoroughly with the Substrate Solution.

VII. ASSAY PROCEDURE

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

1. Prepare all reagents and working standards as directed in the previous sections.
2. Remove excess microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, and reseal.
3. 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 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 200 µL of human IL-1β 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 Substrate Solution to each well. Incubate for 30 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 30 minutes, using a microplate reader set to 450 nm. If wavelength correction is available, set to 540 nm or 570 nm. If wavelength correction is not available, subtract readings at 540 nm or 570 nm from the readings at 450 nm. This subtraction will correct for optical imperfections in the plate. Readings made directly at 450 nm without correction may be higher and less accurate.
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-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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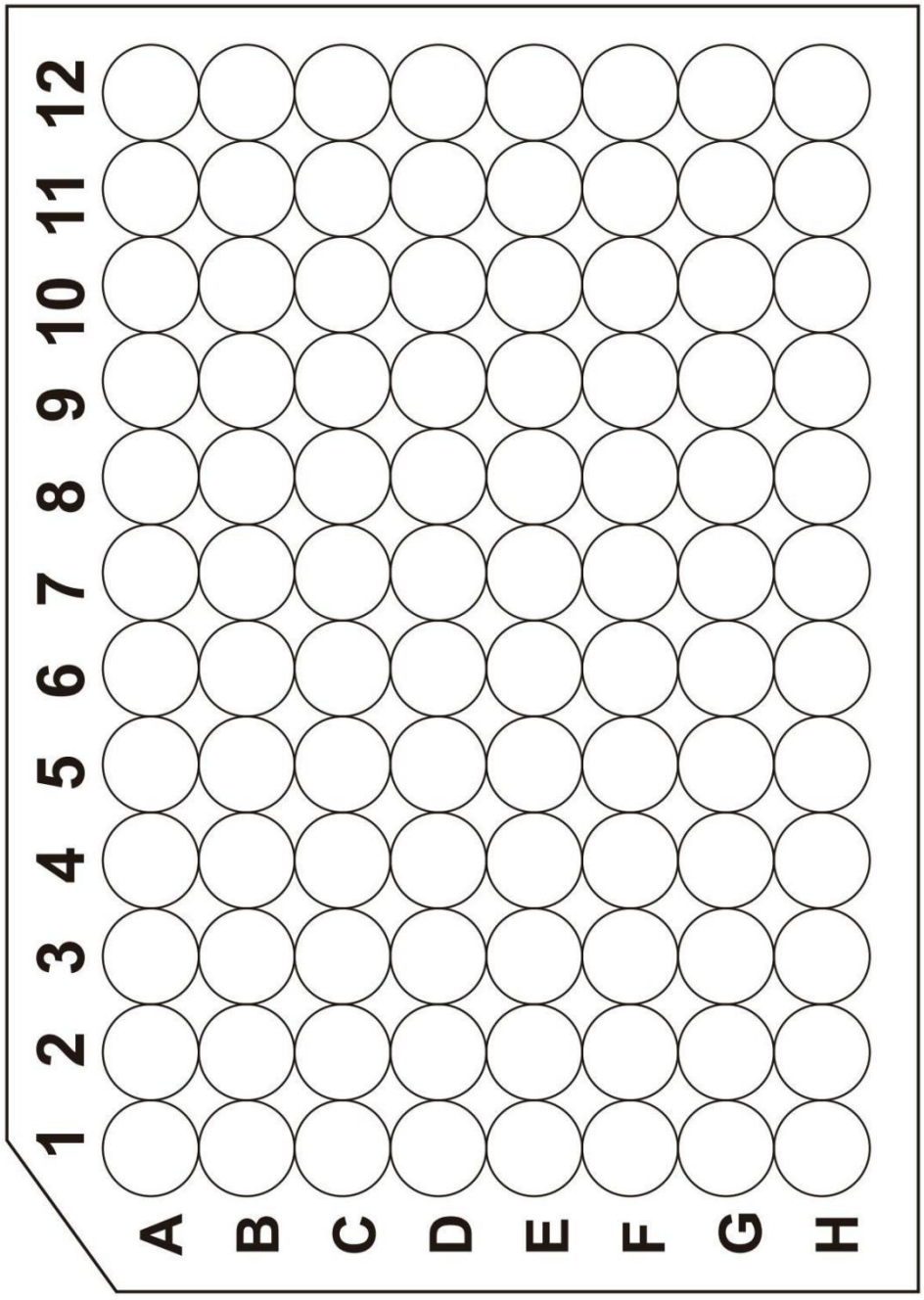
PLATE LAYOUT

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）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-1（IL-1）蛋白家族成员中包括经典的IL-1α和IL-1β。IL-1受体拮抗剂（IL-1RA）、IL-18、IL-33、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-1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单抗包被于微孔板上，样品和标准品中的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多抗，未结合的抗体被洗去；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、人血清和血浆样本；
- 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)	20.7	65.0	141	21.4	66.9	137
标准差	1.20	4.0	5.3	2.3	6.1	9.3
CV%	5.8	6.2	3.8	10.5	9.2	6.8

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
细胞培养上清 (n=4)	97	85 - 107%
血清 (n=4)	89	84 - 92%
血浆 (n=4)	94	93 - 97%

C. 灵敏度

人IL-1β的最低可测剂量（MDD）一般小于1.0pg/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试剂盒经由R&D Systems生产的大肠杆菌表达的高纯度重组人IL-1β蛋白所校正。

E. 线性

在不同类型样本中掺入高浓度的人IL-1β，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=4)	血浆（n=4)
1:2	平均值/期待值（%）	102	99	96
	范围（%)	98 - 107	98 - 101	92-100
1:4	平均值/期待值（%）	103	102	101
	范围（%)	101 - 110	101 - 103	97-105
1:8	平均值/期待值（%）	106	102	105
	范围（%)	103 - 110	101 - 102	103-110
1:16	平均值/期待值（%）	107	105	104
	范围（%)	105 - 113	104 - 106	97-109

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；按下表中所列加不同刺激剂刺激细胞。取细胞培养上清液测定IL-1β含量，结果见表。

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

血清样本 - 使用本试剂盒检测了4份人血清样本中IL-1β的水平。4份样本的检测值均低于最低标准品，3.9pg/mL。

血浆样本 - 使用本试剂盒检测了4份人血浆样本中IL-1β的水平。4份样本的检测值在20.0 - 47.1pg/mL之间，平均值为32.3pg/mL。

G. 特异性

此ELISA法可检测天然及重组人IL-1β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-1β的交叉反应。将50ng/mL干扰因子掺入中间浓度重组人IL-1β控制品中，来检测对人IL-1β的干扰。没有观察到明显的交叉反应或干扰。

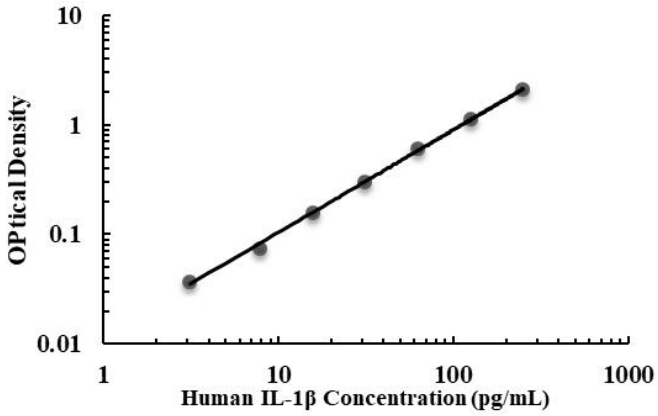
重组人蛋白			重组小鼠蛋白	
EGF	IL-2	IL-9	IL-1α	IL-13
G-CSF	IL-2 sRα	IL-10	IL-1β	TNF-α
GM-CSF	IL-3	IL-11	IL-3	
sgp130	IL-3 sRα	IL-12	IL-4	
GROα	IL-4	IL-13	IL-5	
GROβ	IL-4 sR	IL-18	IL-5 sRα	
IFN-γ	IL-5	PDGF-AA	IL-6	
IGF-I	IL-5 sRβ	PDGF-AB	IL-7	
IGF-II	IL-6	PDGF-BB	IL-9	
IL-1a	IL-6sR	VEGF	IL-10	
IL-1ra	IL-7			

重组人IL-1 sRI和IL-1 sRII在这个测试中没有交叉反应。但当蛋白浓度大于10000pg/mL时，可以观察到干扰。

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	OD	Average	Corrected
0	0.044 0.047	0.046	-
3.1	0.081 0.084	0.083	0.037
7.8	0.120 0.121	0.121	0.075
15.6	0.197 0.212	0.205	0.159
31.3	0.346 0.350	0.348	0.303
62.5	0.655 0.656	0.656	0.610
125	1.202 1.131	1.167	1.121
250	2.097 2.155	2.126	2.081

V. 试剂盒组成及储存

A. 试剂盒组成

组成	描述	规格
人IL-1β Microplate	包被抗体的96孔聚苯乙烯板，8孔×12条	1块板
人IL-1β Conjugate	酶标检测人IL-1β抗体	1瓶
人IL-1β 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
Color Reagent A	显色液A	1瓶
Color Reagent B	显色液B	1瓶
Stop Solution	终止液	1瓶
Plate 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="7">已打开，稀释或重溶的试剂</td><td>洗涤缓冲液（1×）</td><td rowspan="5">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>显色剂A</td></tr>
<tr><td>显色剂B</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><td>将未用的板条放回带有干燥剂的铝箔袋内，密封；2-8℃储存，最多30天*</td></tr>
<tr><td>包被的微孔板条</td></tr>
</table>

*必须在试剂盒有效期内

C. 实验所需自备试验器材

- 酶标仪（可测量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，使用时请做好眼镜、手、面部及衣服的防护。
- 人唾液中含有达到检测浓度的IL-1β。实验时请带口罩，防止唾液污染试剂。

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×）2倍稀释后进行检测，即100μL血清+100μL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℃。

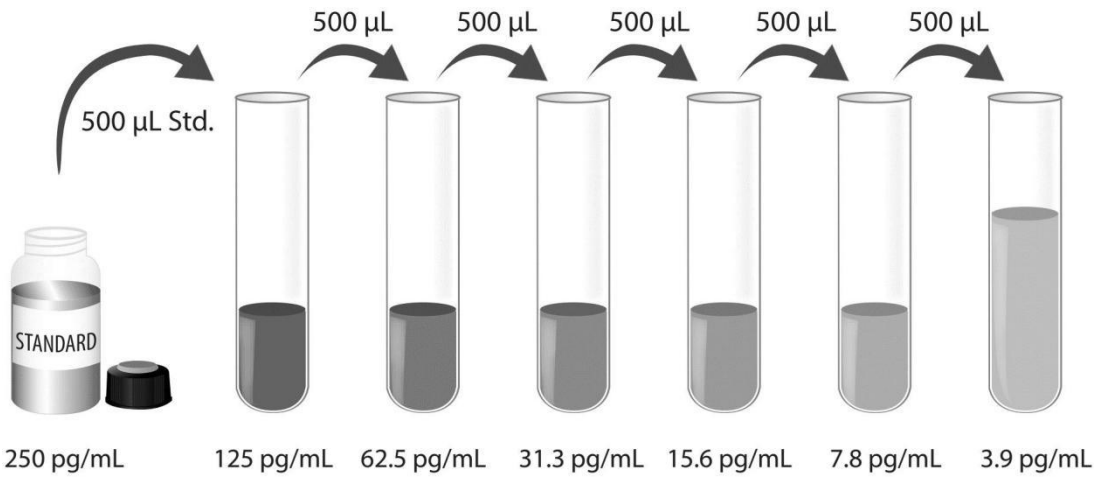
显色剂：按当次试验所需要用量将显色剂A和显色剂B等体积混合，避光；在使用前15分钟准备，仅供当日使用；每孔需200μL。

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250pg/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。

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（250pg/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；
- 混合后的显色底物在上板前应为无色，请避光保存；加入微孔板后，将由无色变成不同深度的蓝色；
- 终止液上板顺序应同显色底物上板顺序一致；加入终止液后，孔内颜色由蓝变黄； 若孔内有绿色，则表明孔内液体未混匀，请充分混合。

VII.操作步骤

使用前请将所有试剂和样本放置于室温，建议所有的实验样本和标准品做复孔检测

1. 按照上一节的说明，准备好所有需要的试剂和标准品；
2. 从已平衡至室温的密封袋中取出微孔板，未用的板条请放回铝箔袋内，重新封口；
3. 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显色底物，室温孵育30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30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