PRODUCT INFORMATION & MANUAL

Mouse IL-1β Valukine™ ELISA

VAL601

For the quantitative determination of natural and
recombinant mouse IL-1β concentrations

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

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Novus kits are guaranteed for 3 months from date of receipt
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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-1F5-10. 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 mice 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-1β precursor by Caspase-1/ICE is a key step in the inflammatory response (2, 8). Neither IL-1α nor IL-1β contains 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-1rα. 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 217 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 mouse IL-1β has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any mouse IL-1β present is bound by the immobilized antibody. After washing away any unbound substances, an enzyme-linked polyclonal antibody specific for mouse 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 mouse 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 and serum.
- 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.

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.
- 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.

III. ADVANTAGES

A. PRECISION

Intra-assay Precision (Precision within an assay)

Two 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	1	2	3
n	20	20	20	20	20
Mean (pg/mL)	37.1	502	27.9	7.9	432
Standard Deviation	1.6	29.4	3.1	7.9	31.1
CV%	4.4	5.9	11.0	9.6	7.2

B. RECOVERY

The recovery of mouse IL-1β spiked to different levels throughout the range of the assay in cell culture media was evaluated. The recovery ranged from 69-102% with an average of 77%.

The recovery of mouse IL-1β spiked to different levels throughout the range of the assay in mouse serum was evaluated. The recovery ranged from 83.2-97.4% with an average of 88.1%.

C. SENSITIVITY

The minimum detectable dose (MDD) of mouse IL-1β is typically less than 3.8 pg/mL.

The MDD was determined by adding two standard deviations to the mean optical density value of twenty zero standard replicates and calculating the corresponding concentration.

D. CALIBRATION

This immunoassay is calibrated against highly purified *E. coli*-expressed recombinant mouse IL-1β produced at R&D Systems®.

E. LINEARITY

To assess the linearity of the assay, different samples were containing or spiked with high concentrations of mouse IL-1β and diluted with Diluent 1× to produce samples with values within the dynamic range of the assay.

Dilution	Average % of Expected	Range (%)
1:2	102	98 - 107
1:4	103	101 - 107
1:8	106	103 - 110
1:16	107	105 - 113

F. SAMPLE VALUES

Cell Culture Supernates - J774A.1 cells were seeded at 3.5 x 10^6 cell/mL and incubated for 6 days in DMEM supplemented with 10% fetal bovine serum, 2.5 µg/mL LPS, and 100 ng/mL rmGM-CSF. The cell culture supernate was assayed for mouse IL-1β and measured 898 pg/mL.

Serum - Four Mouse serum samples were evaluated for the presence of IL-1β in this assay. All samples measured below the lowest standard, 12.5pg/mL.

G. SPECIFICITY

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

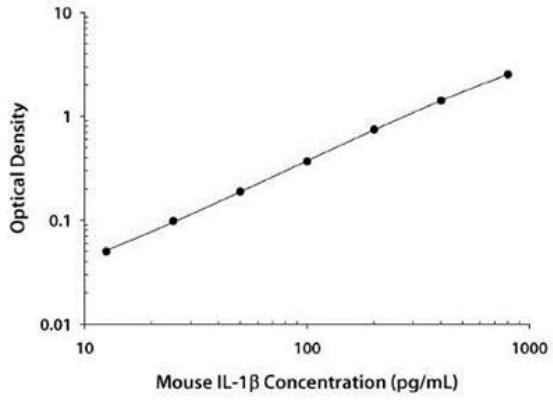
Recombinant Mouse

IL-1α
IL-1Ra
IL-1R1
IL-1R2
IL-1R6
IL-1R9
IL-1Hy2
IL-3
IL-4
IL-5
IL-9

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	0.040 0.042	0.041	—
12.5	0.090 0.091	0.091	0.050
25	0.133 0.144	0.139	0.098
50	0.226 0.232	0.229	0.188
100	0.408 0.410	0.409	0.368
200	0.777 0.789	0.783	0.742
400	1.422 1.476	1.449	1.408
800	2.489 2.611	2.550	2.509

V. KIT COMPONENTS AND STORAGE

A. MATERIALS PROVIDED

Parts	Description	Size
IL-1β Microplate	96 well polystyrene microplate (12 strips of 8 wells) coated with a mouse monoclonal antibody against human IL-1β	1 plate
IL-1β Conjugate	Solution of polyclonal antibody against IL-1β conjugated to horseradish peroxidase	1 vial
IL-1β Standard	recombinant human IL-1β in a buffered protein base; lyophilized	1 vial
Calibrator Diluent (5×)	A 5× 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	Diluted hydrochloric acid	1 vial
Plate Covers	Adhesive strip	3 strips

B. STORAGE

<table>
<tr><td>Unopened Kit</td><td colspan="2">Store at 2-8°C. Do not use past kit expiration date.</td></tr>
<tr><td rowspan="8">Opened/ Reconstituted Reagents</td><td>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 oil ouch 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.

B. SAMPLE PREPARATION

Serum samples require a 5-fold dilution. A suggested 5-fold dilution is 40 µL of sample + 160 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.

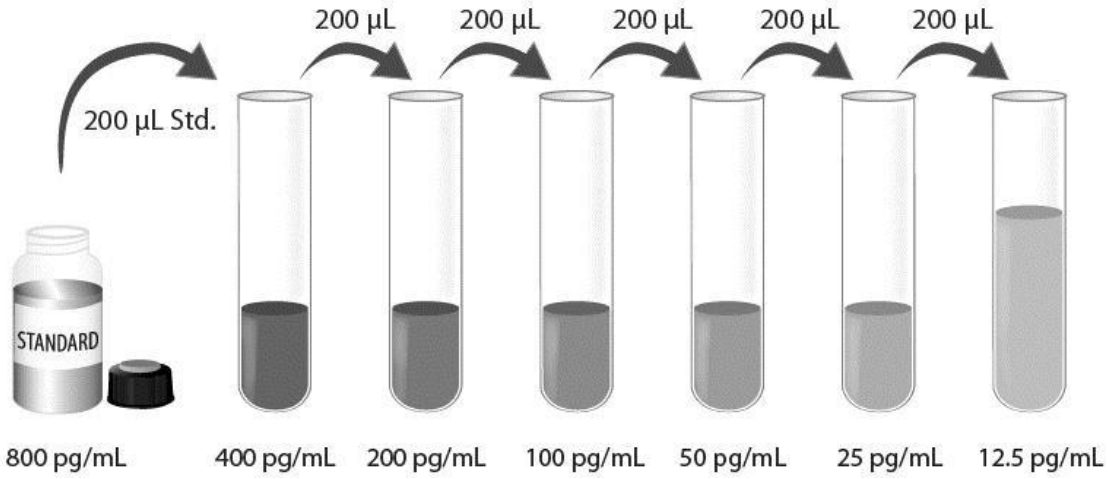
Substrate Solution - Color Reagent A and B should be mixed together in equal volumes within 15 minutes of use. Protect from light. 100 µL of the resultant mixture is required per well.

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

Mouse IL-1β Standard - Refer to the vial label for reconstitution volume*. This reconstitution produces a stock solution of 800 pg/mL. Allow the standard to sit for a minimum of 5 minutes with gentle agitation prior to making dilutions.

*if you have any question, please seek help from our Technical Support.

Pipette 200 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 undiluted standard 800 pg/mL 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 50 µL of Diluent 1× to each well.
4. Add 50 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.
5. 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.
6. Add 100 µL of mouse IL-1β conjugate to each well. Cover with a new adhesive strip. Incubate for 2 hours at room temperature.
7. Repeat the aspiration/wash as in step 5.
8. Add 100 µL of Substrate Solution to each well. Incubate for 30 minutes at room temperature. **Protect from light**.
9. Add 100 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.
10. 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.
11. **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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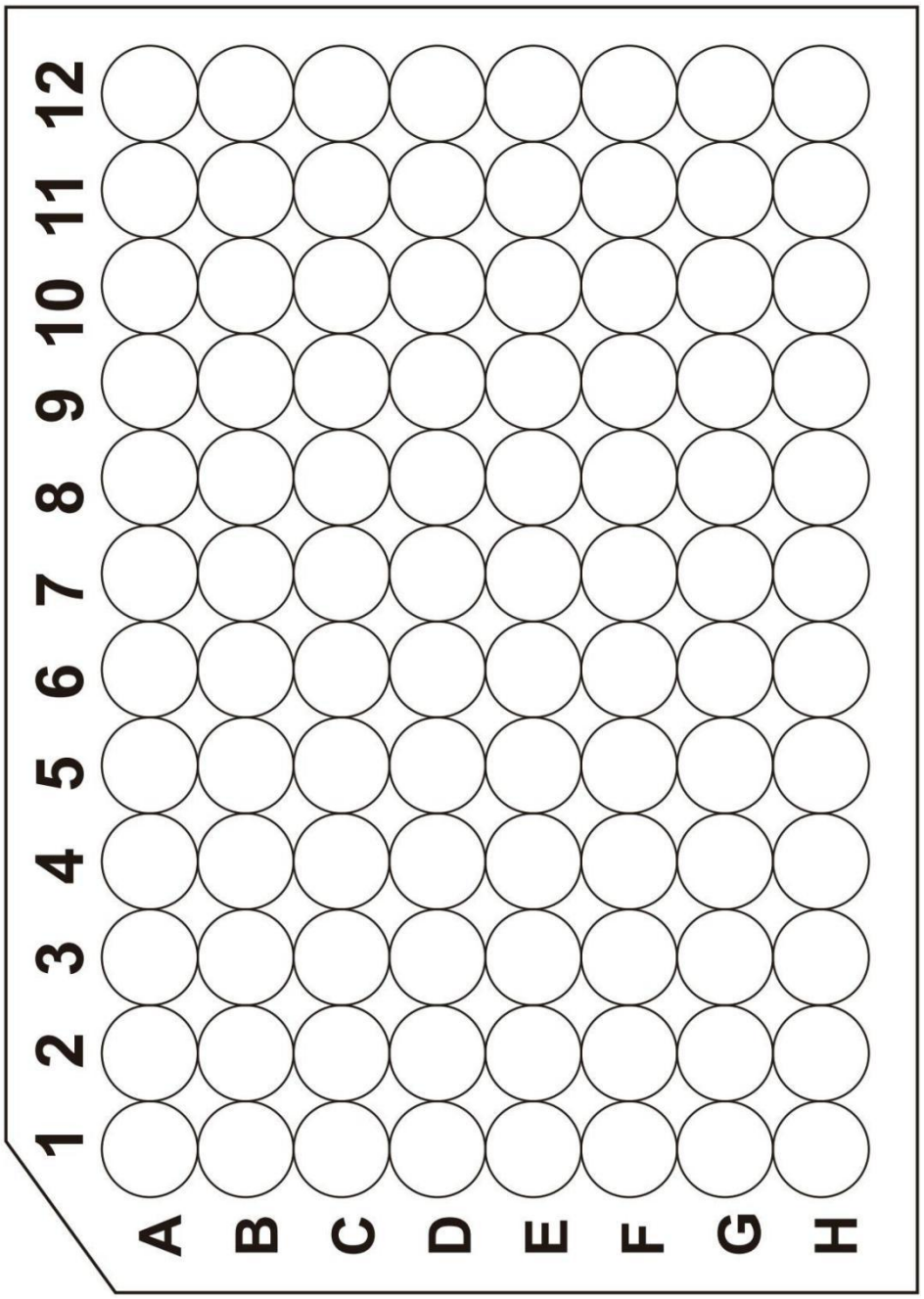
PLATE LAYOUT

Use this plate layout to record standards and samples assayed.



产品信息及操作手册

小鼠 IL-1β Valukine™ ELISA 试剂盒

目录号：VAL601

适用于定量检测天然和重组小鼠 IL-1β 的含量

科研专用，不可用于临床诊断

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Novus 试剂盒确保在你收货日期 3 个月内有效

目录

I. 背景

白细胞介素 1（IL-1）蛋白家族包含经典成员IL-1α、IL-1β 和IL-1rα，以及IL-18、IL-33 和IL-1F5-10。IL-1α和IL-1β结合于相同的细胞表面受体，共享生物学功能（1）。除了皮肤角质形成细胞、一些上皮细胞以及中枢神经系统的某些细胞之外，健康小鼠未刺激的细胞不产生IL-1。 但是，作为对炎症物质、感染或微生物内毒素的应答，巨噬细胞以及其他多种细胞会大量产生 IL-1。在免疫应答与炎症反应、骨重建、发热、糖代谢、生长激素/ IGF-1 的生理学过程中，IL-1β 起着关键作用。在一系列的病理条件下， 包括败血症、类风湿性关节炎、炎性肠病、急性和慢性髓性白血病、胰岛素依赖型糖尿病、动脉粥样硬化、神经损伤以及衰老有关的疾病，一直伴有 IL-1 的异常或持续产生（2-5）。

IL-1α 和IL-1β 具有结构上相关的多肽，在氨基酸水平上的同源性大约为 25%。它们均被合成为 31 kDa 大小的前体，然后裂解为成熟的蛋白质，分子量大约为 17.5 kDa（6, 7）。Caspase-1/ICEI 裂解IL-1β前体是炎症反应中的一个关键步骤（2, 8）。IL-1α 和IL-1β 都不含有典型的疏水性信号肽（9-11），但有证据表明这些因子可通过非经典通路分泌（12, 13）。一部分未加工的 IL-1α 可在细胞膜上出现，并可能存有生物学活性（14）。与IL-1α 前体不同，IL-1β 前体与成熟体相比几乎不具备生物学活性（13, 15）。未加工和成熟的IL-1β 均可从细胞输出。

IL-1α 和IL-1β 通过与IL-1rα结合的免疫球蛋白超家族受体发挥作用。表达 80 kDa 的跨膜I 型受体（IL-1 RI）的细胞包括：T 细胞、成纤维细胞、角质细胞、内皮细胞、滑液内层细胞、软骨细胞和肝细胞（16, 17）。表达 68 kDa 的跨膜II 型受体（IL-1 RII） 的细胞包括：B 细胞、中性粒细胞和骨髓细胞（18）。这两种 IL-1 受体的胞外域具有大约 28%的同源性，但显著差异则体现在其胞内域；II 型受体的胞内域仅有 29 个氨基酸，而I 型受体的胞内域却有 217 个氨基酸。IL-1 RII 对于IL-1 不产生信号，可能做一个诱骗受体减弱IL-1 功能（19）。IL-1 受体辅助蛋白（IL-1 RAcP）与 IL-1 RI 相关，为IL-1 RI 信号转导所必需（20）。IL-1ra 是一个分泌性分子，起到 IL-1 竞争性抑制剂的作用（21, 22）。在人血浆、关节滑液以及多种人细胞系的条件培养基中可以检出可溶性 IL-1 RI 和IL-1 RII。此外，类似于可溶性 IL-1 RII 的IL-1 结合蛋白能够由牛痘和牛痘病毒编码产生（25）。

II. 概述

A. 检测原理

本实验采用双抗体夹心 ELISA 法。抗小鼠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小鼠IL-1β多抗，未结合的抗体被洗去；加入底物溶液（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样本和小鼠血清；
- 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。

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；
- 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。

III. 优势

A. 精确度

板内精确度（同一板内不同孔间的精确度）

已知浓度的两个样本，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	1	2	3
平均值 (pg/mL)	37.1	502	27.9	82.7	432
标准差	1.6	29.4	3.1	7.9	31.1
CV%	4.4	5.9	11	9.6	7.2

B. 回收率

在细胞培养基样本中掺入检测范围内不同水平的小鼠IL-1β，测定其回收率。回收率范围在69-102%，平均回收率在 77%。

在小鼠血清样本中掺入检测范围内不同水平的小鼠IL-1β，测定其回收率。回收率范围在83.2-97.4%，平均回收率在 88.1%。

C. 灵敏度

小鼠IL-1β 的最低可测剂量（MDD）一般小于3.8 pg/mL。

MDD 是根据 20 个标准曲线零点吸光值的平均值加两倍标准差计算得到的相对应浓度。

D. 校正

此 ELISA 试剂盒经 R&D Systems®生产的大肠杆菌表达的高纯度重组小鼠IL-1β 蛋白所校正。

E. 线性

不同的样本中含有或掺入高浓度的小鼠IL-1β，然后用稀释剂（1×）将样本稀释到检测范围内，测定其线性。

稀释倍数	平均值/期待值（%）	范围 （%）
1:2	102	98 - 107
1:4	103	101 - 107
1:8	106	103 - 110
1:16	107	105- 113

F. 样本预值

细胞培养上清液- 培养在含有 10%胎牛血清的DMEM 培养基中J774A.1细胞，用 2.5µg/mL LPS 和 100 ng/mL rmGM-CSF 来刺激。细胞铺板浓度为 3.5×10^5 细胞/瓶。培养 6天后，取上清液，测得 IL-1β的量为 898 pg/mL。

血清样本 - 使用本试剂盒检测了 4 份鼠血清样本中IL-1β 的水平。4份样本的检测值均低于最低标准品，12.5 pg/mL。

G. 特异性

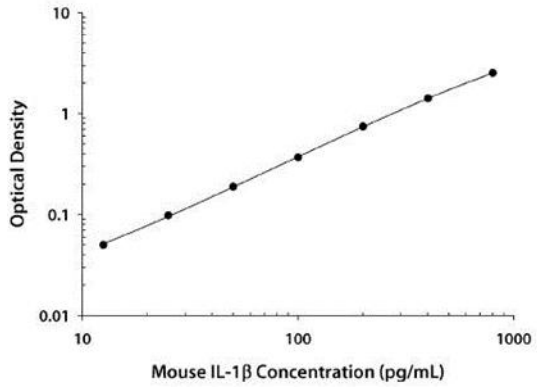
此 ELISA 法可检测天然及重组小鼠 IL-1β 蛋白。将以下因子用稀释剂（1×）配置成 50ng/mL 的浓度来检测与小鼠 IL-1β 的交叉反应。将 50 ng/mL 的干扰因子掺入中间范围的重组小鼠IL-1β 对照品中，来检测对小鼠 IL-1β 的干扰。没有观察到明显的交叉反应或干扰。

重组小鼠蛋白	
IL-1α	IL-1Hy2
IL-1Ra	IL-3
IL-1R1	IL-4
IL-1R2	IL-5
IL-1R6	IL-9
IL-1R9	

IV. 实验标准

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	0.040 0.042	0.041	—
12.5	0.090 0.091	0.091	0.050
25	0.133 0.144	0.139	0.098
50	0.226 0.232	0.229	0.188
100	0.408 0.410	0.409	0.368
200	0.777 0.789	0.783	0.742
400	1.422 1.476	1.449	1.408
800	2.489 2.611	2.550	2.509

V. 试剂盒组成及储存

A. 试剂盒组成

组成	描述	规格
小鼠 IL-1β Microplate	包被抗体的 96 孔聚苯乙烯板，8 孔 × 12 条	1 块板
小鼠 IL-1β Conjugate	酶标检测IL-1β 抗体	1 瓶
小鼠 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 Covers	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="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>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></tr>
<tr><td>标准品</td><td>分装，-20℃以下储存 30 天*；避免反复冻融。</td></tr>
<tr><td>包被的微孔板条</td><td>将未用的板条放回带有干燥剂的铝箔袋内，密封；2-8℃储存，最多 30 天*</td></tr>
</table>

*必须在试剂盒有效期内

C. 实验所需自备试验器材

- 酶标仪（可测量 450 nm 检测波长的吸收值及 540 nm 或 570 nm 校正波长的吸收值）
- 高精度加液器及一次性吸头
- 蒸馏水或去离子水
- 洗瓶（喷瓶）、多通道洗板器或自动洗板机
- 500 mL 量筒

D. 注意事项

- 试剂盒中的终止液是酸性溶液，使用时请做好眼镜、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1000 x 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。

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40 µL血清+160 µL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20 mL 浓缩洗涤液用蒸馏水或去离子水稀释配置成 500 mL 工作浓度的洗涤液。未用完的放回 4℃。

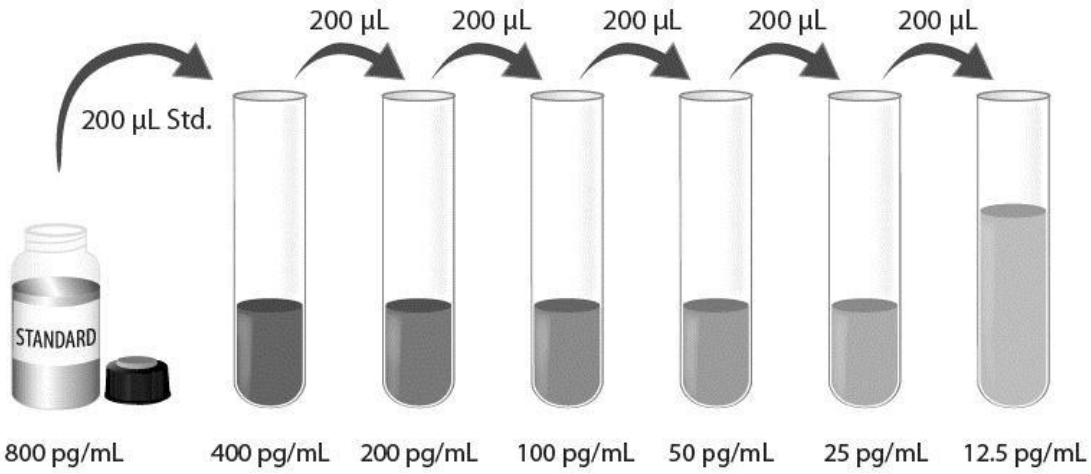
显色剂：按当次试验所需要用量将显色剂 A 和显色剂B 等体积混合，避光；在使用前 15 分钟内准备，仅供当日使用；每孔需 100 µL。

稀释剂（1×）：可将 20mL 浓缩稀释剂（5×）用 80 mL 蒸馏水或去离子水稀释配置成 100 mL 工作浓度的稀释剂。

标准品：参照标准品瓶身注明的方式重溶冻干标准品，得到浓度为800 pg/mL 标准品母液。轻轻震摇至少 5 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 200 µL 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（800 pg/mL），稀释剂（1×）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. 在每个微孔中加入 50 µL 稀释剂（1×）；
4. 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 50 µL。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；
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 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 100 µL 酶标检测抗体。用封板胶纸封住反应孔，室温孵育 2 小时；
7. 重复第 5 步洗板操作；
8. 在每个微孔内加入 100 µL 显色底物，室温孵育 30 分钟。注意避光；
9. 在每个微孔内加入 100 µL 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；
10. 加入终止液后 30 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。如果没有使用双波长校正，结果准确度可能会受影响；
11. **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