PRODUCT INFORMATION & MANUAL

Mouse IL-1β/IL-1F2 Valukine™ ELISA

Catalog Number: VAL601

For the quantitative determination of natural and recombinant mouse Interleukin 1 beta (IL-1β) 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 202609.6

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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-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 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 antibody specific for mouse IL-1β is added to the wells. Following a wash to remove any unbound antibody-enzyme reagent, TMB substrate solution (Chromogenic agent) 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 supernates, tissue lysates, 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 Calibrator Diluent RD5-16 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)

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 forty separate assays to assess inter-assay precision.

	Intra-assay Precision			**Inter-assay Precision**		
Sample	1	2	3	1	2	3
Mean (pg/mL)	33.5	92.9	386	34.6	93.5	423
Standard Deviation	2.5	4.3	11.7	2.9	6.2	23.9
CV%	7.5	4.6	3.0	8.4	6.6	5.7

B. RECOVERY

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

Sample Type	**Average % Recovery**	**Range (%)**
Cell culture supernates (n=4)	106	95-119
Tissue lysates (n=4)	115	110-120
Serum (n=4)	105	98-113
EDTA plasma (n=4)	108	101-117
Heparin plasma (n=4)	97	87-114

SENSITIVITY

Fifty-six assays were evaluated and the minimum detectable dose (MDD) of mouse IL-1β ranged from 0.46-4.80 pg/mL. The mean MDD was 2.31 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.

C. CALIBRATION

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

D. LINEARITY

To assess the linearity of the assay, different samples were containing or spiked with high concentrations of mouse IL-1β and diluted with Calibrator Diluent RD5-16 to produce samples with values within the dynamic range of the assay.

		Cell culture supernates (n=2)	**Tissue lysates (n=2)**	**Serum (n=4)**	**EDTA plasma (n=4)**	**Heparin plasma (n=4)**
1:2	Average % of Expected	98	103	103	99	107
	Range (%)	94-102	100-106	99-104	98-101	98-113
1:4	Average % of Expected	94	96	107	101	108
	Range (%)	94-95	94-98	103-111	98-106	100-113
1:8	Average % of Expected	94	101	110	100	110
	Range (%)	91-96	96-105	107-113	97-103	106-115
1:16	Average % of Expected	88	96	111	98	108
	Range (%)	85-91	96-97	110-111	94-102	101-119

E. SAMPLE VALUES

Serum/Plasma - Twenty samples were evaluated for the presence of mouse IL-1β in this assay. Serum samples measured as shown in the table below. All twenty plasma samples measured less than the lowest Mouse IL-1β Standard, 12.5 pg/mL.

Sample Type	Mean of Detectable (pg/mL)	% Detectable	Range (pg/mL)
Serum (n=20)	27.6	10	ND-37.9

ND=Non-detectable

Cell Culture Supernates - J774A.1 mouse reticulum cell sarcoma macrophage cells (3.5×10^6 cells/mL) were cultured in DMEM supplemented with 10% fetal bovine serum and stimulated with 5 µM β-mercapthoethanol, 2 mM L-glutamine, 100 U/mL penicillin, and 100 µg/mL streptomycin sulfate. The cell culture supernate was unstimulated or stimulated with 2.5 µg/mL of lipopolysacharride (LPS) and 100 ng/mL of recombinant mouse GM-CSF (R&D Systems, Catalog # 415-ML) for 3 and 6 days. Aliquots of the cell culture supernates were removed and assayed for levels of mouse IL-1β.

Sample Type	Day 3 (pg/mL)	Day 6 (pg/mL)
J774A.1 Unstimulated	ND	ND
J774A.1 Stimulated with LPS + GM-CSF	587	938

ND=Non-detectable

Tissue Lysates - Organs from 2-3 mice were rinsed with PBS to remove excess blood, chopped into 1-2 mm pieces, homogenized with a tissue homogenizer, and 1 mL of Lysis Buffer 2 was added (2 mL of Lysis Buffer 2 was added to liver tissue). Organs were lysed at room temperature for 30 minutes with gentle agitation and centrifuged to remove debris. An aliquot of each tissue lysate was removed and assayed for levels of mouse IL-1β.

Tissue Lysate	(pg/mL)
Brain	20.9
Heart	28.3
Kidney	111
Liver	347
Lung	115
Spleen	987

F. SPECIFICITY

This assay recognizes both natural and recombinant mouse IL-1β.

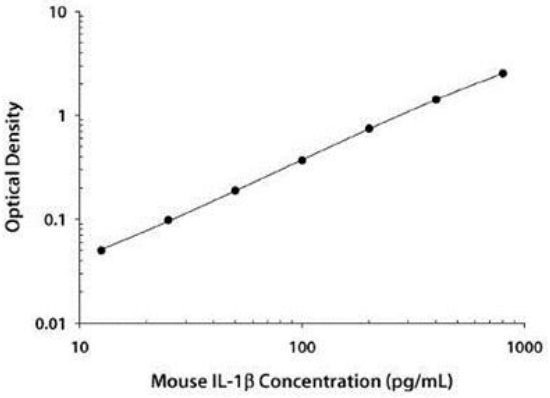
The following factors were prepared at 50 ng/mL and assayed for cross-reactivity. Preparations of the following factors at 50 ng/mL in a mid-range recombinant mouse IL-1β control were assayed for interference. No significant cross-reactivity or interference was observed.

Recombinant mouse:	**Recombinant rat:**	**Other recombinants:**
IL-1α	IL-1α	canine IL-1β
IL-1F10/IL-1HY2	IL-1β	cotton rat IL-1β
IL-1ra		human IL-1β
IL-1 RAPL2/IL-1 R9		human pro-IL-1β
IL-1 RI		porcine IL-1β
IL-1 RII		rhesus macaque IL-1β
IL-1 Rrp2/IL-1 R6		
IL-2		
IL-3		
IL-4		
IL-5		
IL-6		
IL-7		
IL-9		
IL-10		
IL-10 Rα		
IL-11		
IL-11 Rα		
IL-12		
IL-12 p40		
IL-13		
IL-15		
IL-20		
IL-23 R		
ST2/IL-1 R4		

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
Mouse IL-1β Microplate	96 well polystyrene microplate (12 strips of 8 wells) coated with an antibody against mouse IL-1β	1 plate
Mouse IL-1β Conjugate	Solution of an antibody against IL-1β conjugated to horseradish peroxidase	1 vial
Mouse IL-1β Standard	Recombinant mouse IL-1β in a buffered protein base; lyophilized. Refer to the vial label for reconstitution volume.	1 vial
Assay Diluent RD1N	A buffered protein solution with preservatives.	1 vial
Calibrator Diluent RD5-16	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	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="7">Opened/ Reconstituted Reagents</td><td>Wash buffer (1×)</td><td rowspan="5">May be stored for up to 1 month at 2-8°C.*</td></tr>
<tr><td>Assay Diluent RD1N</td></tr>
<tr><td>Calibrator Diluent RD5-16</td></tr>
<tr><td>Stop Solution</td></tr>
<tr><td>Conjugate</td></tr>
<tr><td>TMB Substrate</td><td rowspan="2">Aliquot and store for up to 1 month at -20°C in a manual defrost freezer.* Avoid repeated freeze-thaw cycles.</td></tr>
<tr><td>Standard</td></tr>
<tr><td>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.

If using tissue lysate samples, the following is also required:

- Cell Lysis Buffer 2 (R&D Systems®, Catalog # 895347)

D. PRECAUTION

- Assay Diluent RD1N 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

The sample collection and storage conditions listed below are intended as general guidelines. Sample stability has not been evaluated.

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 RD5-16.

Tissue Lysates - Cells must be lysed before assaying. See Sample Values section for details. Samples may require dilution with Calibrator Diluent RD5-16.

Serum - Allow blood samples to clot for 2 hours at room temperature before centrifuging for 20 minutes at 2000 × 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 RD5-16.

Plasma - Collect plasma using EDTA or heparin as an anticoagulant. Centrifuge for 20 minutes at 2000 × 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 RD5-16.

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

Do not use icteric samples.

B. REAGENT PREPARATION

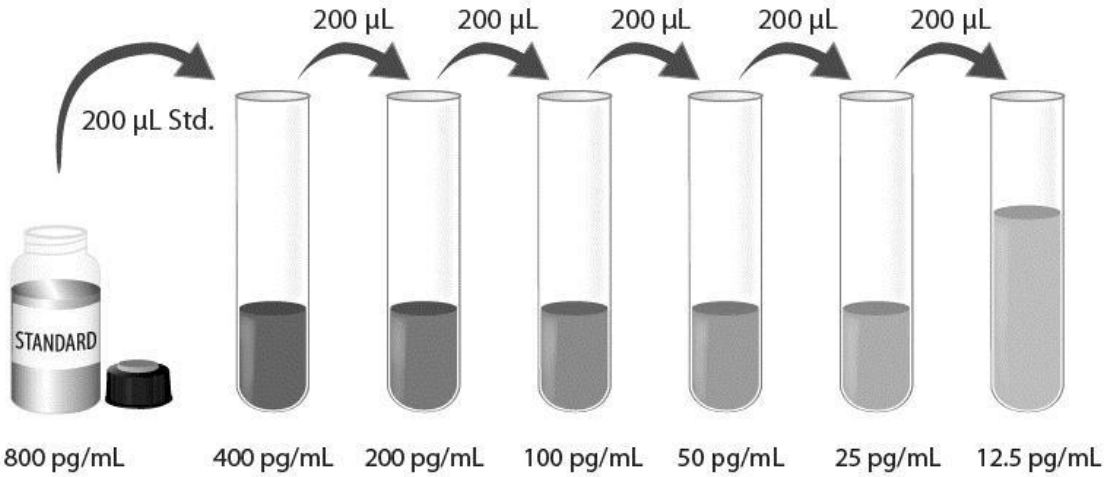
Note: *Bring all reagents to room temperature before use.*

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×).

Mouse IL-1β Standard - Centrifuge briefly before opening. Refer to the vial label for reconstitution volume*. Reconstitute the Mouse IL-1β Standard with Calibrator Diluent RD5-16. Do not substitute other diluents. 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 Calibrator Diluent RD5-16 into each tube. Use the stock solution to produce a dilution series (below). Mix each tube thoroughly before the next transfer. The standard 800 pg/mL serves as the high standard. The Calibrator Diluent RD5-16 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 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 TMB Substrate .

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 Assay Diluent RD1N to each well.
4. Add 50 µL of Standard and 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 four times for a total of five 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 TMB Substrate to each well. **Incubate for 30 minutes at room temperature. Protect from light.**
9. Add 100 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. 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 mouse 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.

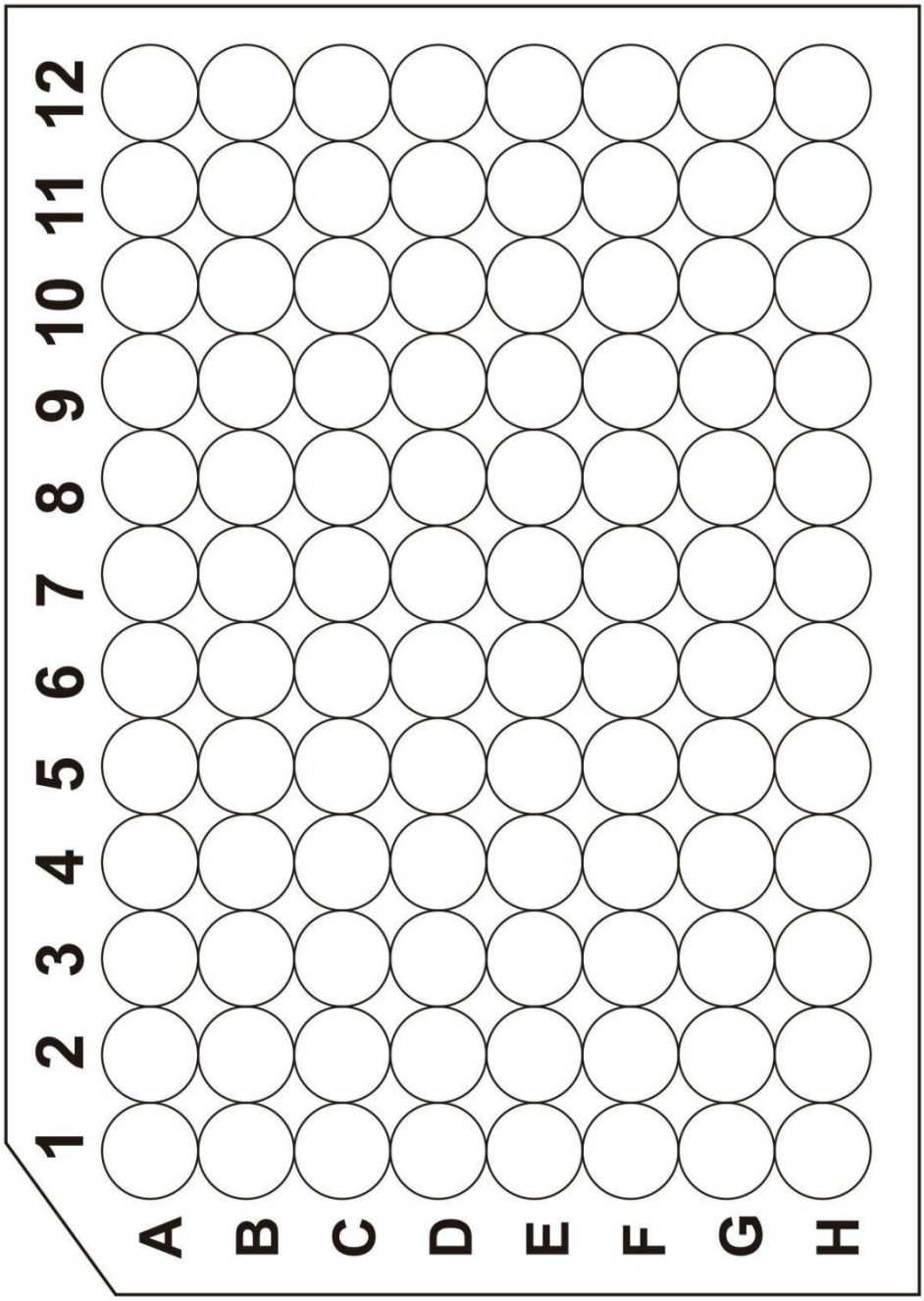
If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.

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白细胞介素 1β (IL-1β)的含量

科研专用，不可用于临床诊断

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版本号 202609.6

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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-1ra，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/ICE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-1ra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213个氨基酸。IL-1 RII对于IL-1不产生信号，可能作为一个诱骗受体减弱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（23, 24）。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抗体包被于微孔板上，样品和标准品中的小鼠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抗体，未结合的抗体被洗去；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样本，组织裂解液，血清和血浆；
- 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RD5-16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40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)	33.5	92.9	386	34.6	93.5	423
标准差	2.5	4.3	11.7	2.9	6.2	23.9
CV%	7.5	4.6	3.0	8.4	6.6	5.7

B. 回收率

对小鼠IL-1β在不同基质中、覆盖整个检测范围的三水平加标回收率进行了评估。

样本类型	平均回收率 (%)	范围 (%)
细胞培养上清 (n=4)	106	95-119
组织裂解液 (n=4)	115	110-120
血清 (n=4)	105	98-113
EDTA 血浆 (n=4)	108	101-117
肝素血浆(n=4)	97	87-114

C. 灵敏度

对56次检测进行了评估，小鼠IL-1β的最低可检测剂量（MDD）范围为0.46-4.80 pg/mL，平均MDD为2.31 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，然后用标准品稀释液RD5-16将样本稀释到检测范围内，测定其线性。

		细胞培养上清 (n=2)	组织裂解液 (n=2)	血清 (n=4)	EDTA血浆 (n=4)	肝素血浆 (n=4)
1:2	平均值/期待值（%）	98	103	103	99	107
	范围（%）	94-102	100-106	99-104	98-101	98-113
1:4	平均值/期待值（%）	94	96	107	101	108
	范围（%）	94-95	94-98	103-111	98-106	100-113
1:8	平均值/期待值（%）	94	101	110	100	110
	范围（%）	91-96	96-105	107-113	97-103	106-115
1:16	平均值/期待值（%）	88	96	111	98	108
	范围（%）	85-91	96-97	110-111	94-102	101-119

F. 样本预值

血清样本 - 本检测评估了二十份样本中mouse IL-1β的存在情况。血清样本的测定结果如下表所示。全部二十份血浆样本的测定值均低于最低的 Mouse IL-1β标准品，即12.5 pg/mL。

样本类型	平均检测值 (pg/mL)	检测率 (%)	范围 (pg/mL)
血清 (n=20)	27.6	10	ND-37.9

ND=未检出

细胞培养上清液 - J774A.1小鼠网状细胞肉瘤巨噬细胞（3.5 × 10^6个/mL）在添加了10%胎牛血清的DMEM中培养，并以5 µM β-巯基乙醇、2 mM L-谷氨酰胺、100 U/mL青霉素和100 µg/mL硫酸链霉素进行刺激。细胞培养上清液分别为未刺激，或用2.5 µg/mL脂多糖（LPS）和100 ng/mL 重组小鼠 GM-CSF（R&D Systems，货号415-ML）刺激3天和6天。取出一部分细胞培养上清液，检测mouse IL-1β的水平。

样本类型	3 天 (pg/mL)	6 天 (pg/mL)
J774A.1 未刺激	ND	ND
J774A.1 用 LPS + GM-CSF 刺激	587	938

ND=未检出

组织裂解液 - 取2-3只小鼠的器官，用PBS冲洗以去除多余血液，切成1-2 mm的小块，用组织匀浆器匀浆，并加入1 mL裂解缓冲液2（肝组织则加入2 mL裂解缓冲液2）。器官在室温下轻柔振荡裂解30分钟，然后离心以去除碎片。取出一部分各组织裂解液，检测mouse IL-1β的水平。

组织裂解液	(pg/mL)
脑	20.9
心脏	28.3
肾	111
肝	347
肺	115
脾	987

G. 特异性

此ELISA法可检测天然及重组小鼠IL-1β蛋白。

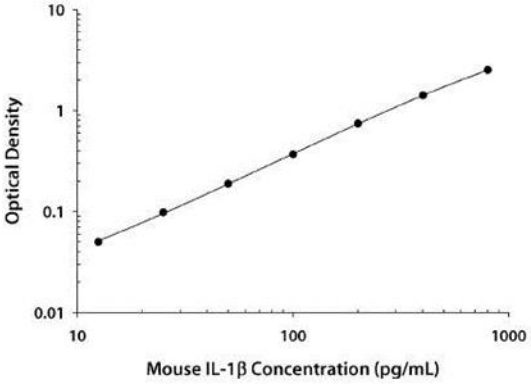
将以下因子配制成50 ng/mL的浓度来检测与小鼠IL-1β的交叉反应。将50 ng/mL的干扰因子掺入中间范围的重组小鼠IL-1β对照品中，来检测对小鼠IL-1β的干扰。没有观察到明显的交叉反应或干扰。

Recombinant mouse:	Recombinant rat:	Other recombinants:
IL-1α	IL-1α	canine IL-1β
IL-1F10/IL-1HY2	IL-1β	cotton rat IL-1β
IL-1ra		human IL-1β
IL-1 RAPL2/IL-1 R9		human pro-IL-1β
IL-1 RI		porcine IL-1β rhesus
IL-1 RII		macaque IL-1β
IL-1 Rrp2/IL-1 R6		
IL-2		
IL-3		
IL-4		
IL-5		
IL-6		
IL-7		
IL-9		
IL-10		
IL-10 Rα		
IL-11		
IL-11 Rα		
IL-12		
IL-12 p40		
IL-13		
IL-15		
IL-20		
IL-23 R		
ST2/IL-1 R4		

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	0.040 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
Mouse IL-1β Microplate	包被抗小鼠 IL-1β抗体的 96 孔聚苯乙烯板，8 孔 × 12 条	1 块板
Mouse IL-1β Conjugate	酶标小鼠 IL-1β检测抗体	1 瓶
Mouse IL-1β Standard	标准品（冻干），参考瓶身标签进行重溶	1 瓶
Assay Diluent RD1N	检测液 RD1N	
Calibrator Diluent RD5-16	标准品稀释液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	1 瓶
Stop Solution	终止液	1 瓶
Plate Covers	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>洗涤液（1×）</td><td rowspan="5">2-8℃储存，最多 30 天*</td></tr>
<tr><td>检测液 RD1N</td></tr>
<tr><td>标准品稀释液 RD5-16</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><td rowspan="2">分装，-20℃以下储存 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

如果使用组织裂解液样本，还需要以下物品：

- 细胞裂解缓冲液2（R&D Systems，货号895347）

D. 注意事项

- 检测液RD1N含有叠氮化钠，可能与铅制和铜制管道发生反应，形成具有爆炸性的金属叠氮化物。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。避免吸入雾气。
- 请佩戴防护手套、防护服、护目镜和面罩。操作后彻底洗手。

VI. 实验前准备

A. 样品收集及储存

以下列出的样本采集和储存条件仅为一般性指南。样本稳定性尚未经过评估。

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RD5-16稀释。

组织裂解液：检测前必须裂解细胞。详情参见样本值部分。样本可能需要用标准品稀释液 RD5-16进行稀释。

血清：让血液样本在室温下凝固2小时，然后以2000 × g 离心20分钟。取出血清后立即检测，或分装后于≤ -20°C储存样本。避免反复冻融。样本可能需要用标准品稀释液RD5-16进行稀释。

血浆：使用EDTA或肝素作为抗凝剂采集血浆。采集后30分钟内以2000 × g 离心20分钟。立即检测，或分装后于≤ -20°C 储存样本。避免反复冻融。样本可能需要用标准品稀释液RD5-16进行稀释。

注意：*枸橼酸盐血浆尚未在本检测中经过验证。*

请勿使用黄疸样本。

B. 检测前准备工作

使用前请将所有试剂放置于室温

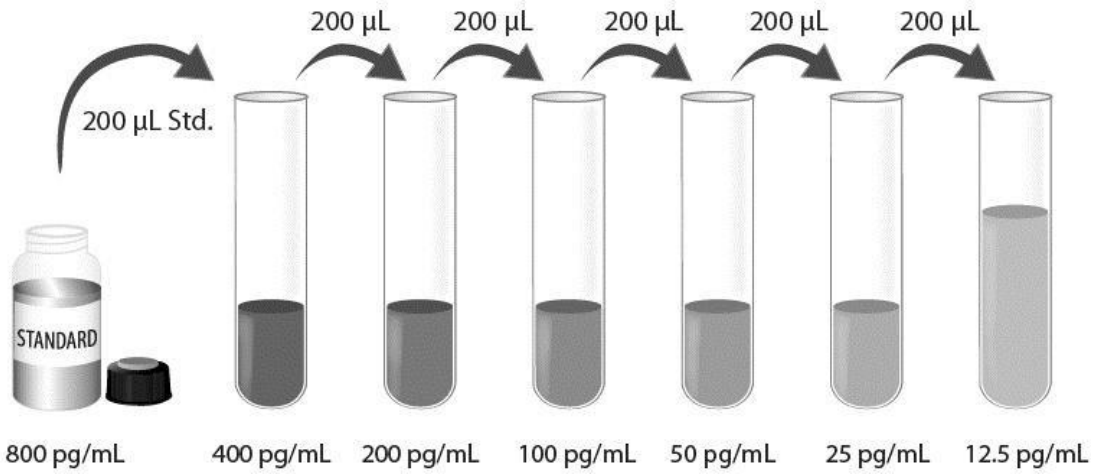
洗涤液（1×）：从冰箱中取出的浓缩洗涤液可能有结晶，属于正常现象；放置室温，轻摇混匀，待结晶完全溶解后再配制洗涤液。可将20 mL浓缩洗涤液用去离子水或蒸馏水稀释配制成500 mL工作浓度的洗涤液（1×）。

小鼠IL-1β标准品：开盖前请瞬时离心。参照标准品瓶身注明的方式重溶冻干标准品*。用标准品稀释液RD5-16复溶 Mouse IL-1β标准品。请勿使用其他稀释液替代。此复溶操作可得到浓度为800 pg/mL 的储备液。在进行稀释之前，让标准品静置至少5分钟并轻柔振荡。

*如有疑问，请咨询我们的技术支持。

每个稀释管中加入200 µL标准品稀释液RD5-16。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。800 pg/mL管作标准曲线最高点，标准品稀释液

RD5-16可用作标准曲线零点（0pg/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

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检测液RD1N；
4. 分别将不同浓度标准品和实验样本加入相应孔中，每孔50 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4次，共洗5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。用封板膜封住反应孔，**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 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100 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。数据可以通过绘制小鼠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。

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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

