PRODUCT INFORMATION & MANUAL

Mouse IL-23 Valukine™ ELISA

Catalog Number: VAL637

For the quantitative determination of natural and recombinant mouse Interleukin 23 (IL-23) 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 202411.1

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I. BACKGROUND

Interleukin 23 (IL-23) is a heterodimeric cytokine that is related to IL-12 (1-3). It is composed of two disulfide-linked subunits, a 19 kDa (p19) subunit that is unique to IL-23, and a 40 kDa (p40, IL-12β) subunit that is shared with IL-12 (3-7). The mouse p19 cDNA encodes a 196 amino acid (aa) precursor with a putative 21 aa signal peptide and 175 aa mature protein with structural similarity to the IL-6 family of single chain cytokines and to the p35 subunit of IL-12. The mouse p40 cDNA encodes a 335 aa precursor with a putative 22 aa signal peptide and 313 aa mature protein that contains four potential glycosylation sites, a C2-type immunoglobulin domain and a fibronectin type III domain. Mature mouse p19 and p40 share 88% and 92% aa sequence identity, respectively, with the corresponding rat subunits. IL-23 is produced by activated macrophages, microglia, and monocyte-derived dendritic cells in response to pathogens including certain bacteria and viruses and/or their components (3, 6). The p19 subunit is also expressed by polarized T cells, but these cells do not express the p40 subunit (6).

The functional IL-23 receptor complex consists of two receptor subunits, the IL-12 receptor β1 subunit (IL-12 Rβ1) and the IL-23-specific receptor subunit (IL-23 R) (7). IL-12 Rβ1 is a 738 aa type I transmembrane glycoprotein with a 546 aa extracellular domain that contains a WSXWS motif and five fibronectin type III domains. IL-23 R is a 644 aa type I transmembrane glycoprotein with a 351 aa extracellular domain that contains two fibronectin type III domains. The IL-23 receptor complex is expressed in mouse Th1 and Th2 cells, bone marrow dendritic cells, IFN-γ-activated macrophages, and $CD4^+$ $CD45RB^{low}$ memory T cells (7). IL-23 initiates a signal transduction cascade similar to that of IL-12, and involves Jak2, Tyk2, STAT1, STAT3, STAT4, and STAT5 (7). IL-23 will bind to IL-12 Rβ1, but signaling requires the presence of the IL-23 R subunit (1-3).

IL-23 and IL-12 have overlapping and distinct biological activities. The IL-23 immune pathway induces the earliest recruitment of neutrophils to the site of infection, while the more classic host defense and cytotoxic response is stimulated by IL-12 (4). IL-12 drives the development of Th1 cells and induces production of IFN-γ by NK cells (3). In contrast, IL-23 has a role in the development and maintenance of a T cell subset, designated Th17, that is characterized by the production of IL-17A, IL-17F, IL-6, and TNF-α (3, 4, 8). The induction of Th17 cells involves the actions of TGF-β, while their

survival and expansion appears to be IL-23-dependent (9-11). The IL-23/IL-17 axis is an important mediator of inflammation. In mouse models, transgenic over-expression of IL-23 leads to a lethal systemic inflammatory response (12). IL-23 effects on Th17 cells may also enhance the development of several models of autoimmune disease including experimental allergic encephalomyelitis (EAE), collagen- induced arthritis (CIA), colitis, and diabetes (5, 8, 13-17). IL-23 may also play a role in increased tumor growth associated with chronic inflammation (18). In humans, IL-23 has been found upregulated in several pathologies with dysregulated immune function including psoriasis, Crohn's disease, and multiple sclerosis (19-21).

II. OVERVIEW

A. PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. An antibody specific for mouse IL-23 p19 subunit has been pre-coated onto a microplate. Standards, control and samples are pipetted into the wells and any mouse IL-23 present is bound by the immobilized antibody. After washing away any unbound substances, an enzyme-linked antibody specific for mouse IL-23 p40 subunit 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 mouse IL-23 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, mouse plasma and mouse 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 Calibrator Diluent RD5-3 and repeat the assay.
- Any variation in operator, pipetting technique, washing technique, incubation time or temperature, and kit age can cause variation in binding.

III. ADVANTAGES

A. PRECISION

Intra-assay Precision (Precision within an assay)

Three samples were tested twenty times on one plate to assess intra-assay precision.

Inter-assay Precision (Precision between assays)

Three samples were tested in forty separate assays to assess inter-assay precision.

	Intra-assay Precision			**Inter-assay Precision**		
Sample	1	2	3	1	2	3
Mean (pg/mL)	49.3	107	344	50.4	116	333
Standard Deviation	3.72	5.84	22.7	3.69	7.14	18.6
CV%	7.5	5.5	6.6	7.3	6.2	5.6

B. RECOVERY

The recovery of mouse IL-23 spiked to three levels throughout the range of the assay in various matrices was evaluated.

Sample Type	**Average % Recovery**	**Range (%)**
Cell culture media (n=4)	103	99-108
Mouse serum (n=4)	95	90-101
Mouse heparin plasma (n=4)	99	88-117

C. SENSITIVITY

Forty assays were evaluated and the minimum detectable dose (MDD) of mouse IL-23 ranged from 0.68-4.17 pg/mL. The mean MDD was 2.28 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 *Sf* 21-expressed

covalently-linked p19 and p40 subunits of recombinant mouse IL-23 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-23 and diluted with Calibrator Diluent RD5-3 to produce samples with values within the dynamic range of the assay.

Dilution		Cell culture samples (n=4)	Mouse serum (n=4)	Mouse heparin plasma (n=4)
1:2	Average % of Expected	98	104	102
	Range (%)	93-101	102-105	97-106
1:4	Average % of Expected	98	107	109
	Range (%)	93-102	104-109	104-115
1:8	Average % of Expected	94	107	110
	Range (%)	88-98	100-110	105-115
1:16	Average % of Expected	93	108	110
	Range (%)	85-97	106-110	105-116

F. SAMPLE VALUES

Mouse serum/ plasma - Samples were evaluated for detectable levels of mouse IL-23 in this assay. No detectable levels were observed.

Cell Culture Supernates - J774A.1 mouse reticulum cell sarcoma macrophage cells were cultured for three days in DMEM supplemented with 10% fetal bovine serum, 2 mM L-glutamine, 100 U/mL penicillin, 100 µg/mL streptomycin sulfate, 25 ng/mL lipopolysaccharide (LPS), and 100 ng/mL recombinant mouse GM-CSF. An aliquot of

the cell culture supernate was removed, assayed for mouse IL-23, and measured 58.6 pg/mL.

G. SPECIFICITY

This assay recognizes natural and recombinant mouse IL-23.

The factors listed below were prepared at 50 ng/mL in Calibrator Diluent RD5-3 and assayed for cross-reactivity. Preparations of the following factors at 50 ng/mL in a mid-range mouse IL-23 control were assayed for interference. No significant cross-reactivity or interference was observed.

Recombinant mouse:	**Recombinant rat:**	**Other recombinants:**
IL-12	IL-12	canine IL-12
IL-12 p35	IL-12 p35	porcine IL-12
IL-12 p40 dimer	IL-23	canine IL-12 p40
IL-12 p40 monomer	**Recombinant human:**	
IL-12 Rβ	IL-23	
IL-23 R	IL-23 p19	
	IL-23 R	

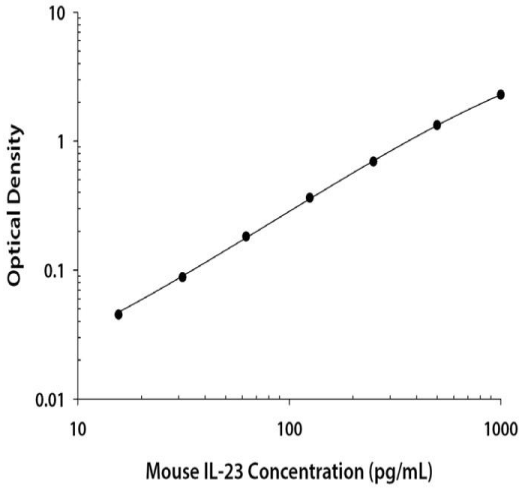
This assay does not recognize free p19 or p40 subunits.

Recombinant mouse IL-23 p19 does not cross-react but does interferes at concentrations ≥ 50 ng/mL in this assay.

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	0.049 0.049	0.049	——
15.6	0.091 0.097	0.094	0.045
31.3	0.137 0.137	0.137	0.088
62.5	0.223 0.238	0.231	0.182
125	0.409 0.415	0.412	0.363
250	0.734 0.749	0.742	0.693
500	1.367 1.389	1.378	1.329
1000	2.299 2.377	2.338	2.289

V. KIT COMPONENTS AND STORAGE

A. MATERIALS PROVIDED

Parts	Description	Size
Mouse IL-23 Microplate	96 well polystyrene microplate (12 strips of 8 wells) coated with an antibody against mouse IL-23 p19 subunit.	1 plate
Mouse IL-23 Conjugate	An antibody specific for mouse IL-23 p40 subunit conjugated to horseradish peroxidase.	1 vial
Mouse IL-23 Standard	Recombinant mouse IL-23 in a buffered protein base; lyophilized. Refer to the vial label for reconstitution volume.	1 vial
Mouse IL-23 Control	Recombinant mouse IL-23 in a buffered protein base; lyophilized. The assay value of the control should be within the range specified on the vial label.	1 vial
Assay Diluent RD1-14	A buffered protein solution. *(May contain a precipitate. Mix well before and during use).*	1 vial
Calibrator Diluent RD5-3	A buffered protein solution used to dilute standard and samples.	1 vial
Wash Buffer Concentrate (25×)	A 25× concentrated solution of buffered surfactant.	1 vial
TMB Substrate	TMB ELISA Substrate Solution/TMB Substrate Solution.	2 vials
Stop Solution	Diluted hydrochloric 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>Assay Diluent RD1-14</td></tr>
<tr><td>Stop Solution</td></tr>
<tr><td>Conjugate</td></tr>
<tr><td>TMB Substrate</td></tr>
<tr><td>Calibrator Diluent RD5-3</td></tr>
<tr><td>Control</td><td rowspan="2">May be stored for up to 1 month at 2-8°C.*</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.
- Horizontal orbital microplate shaker (0.12" orbit) capable of maintaining a speed of 500 ± 50 rpm.
- Polypropylene test tubes for dilution of standards and samples.

D. PRECAUTION

- Some components in this kit contain a preservative which may cause an allergic skin reaction. Avoid breathing mist.
- The Stop Solution provided with this kit is an acid solution. Wear eye, hand, face, and clothing protection when using this material.

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 RD5-3.

Serum - Allow blood samples to clot for 2 hours at room temperature before centrifuging for 20 minutes at 2000 x g. Remove serum and assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles. Samples may require dilution with Calibrator Diluent RD5-3.

Plasma - Collect plasma using heparin as an anticoagulant. Centrifuge for 20 minutes at 2000 x g within 30 minutes of collection. Assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles. Samples may require dilution with Calibrator Diluent RD5-3.

Note: *EDTA plasma is not recommended for use in this assay.*

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

Hemolyzed and icteric samples are not suitable for use in this assay.

B. REAGENT PREPARATION

Bring all reagents to room temperature before use.

Mouse IL-23 Control - Reconstitute the control with 1.0 mL of deionized or distilled water. Mix thoroughly. Assay the control undiluted.

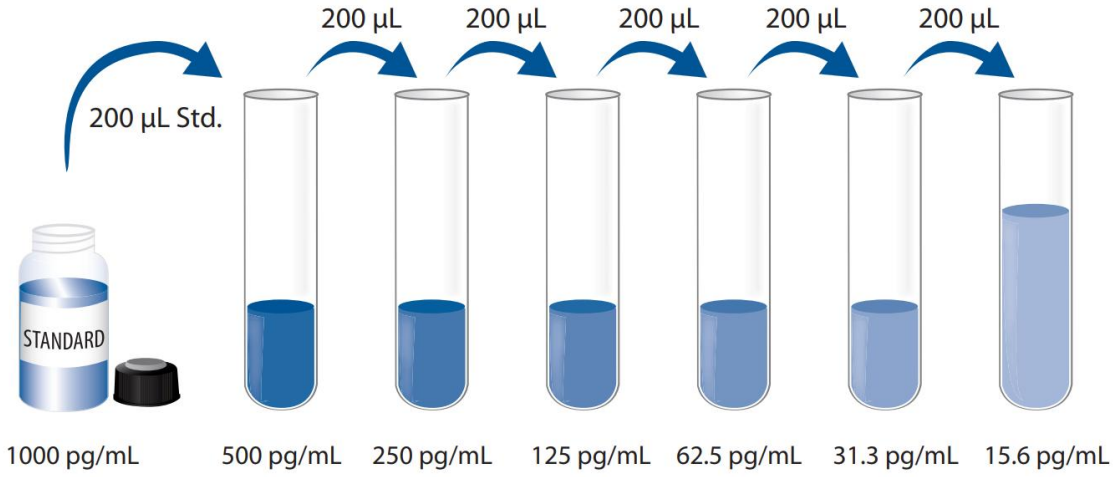
Wash Buffer (1×) - If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. Dilute 20 mL of Wash Buffer Concentrate (25×) into deionized or distilled water to prepare 500 mL of Wash Buffer (1×).

Mouse IL-23 Standard- Refer to the vial label for the reconstitution volume* Reconstitute the Mouse IL-23 Standard with Calibrator Diluent RD5-3. Do not substitute other diluents. This reconstitution produces a stock solution of 1000 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.

Use polypropylene tubes. Pipette 200 µL of Calibrator Diluent RD5-3 into each tube. Use the standard stock solution to produce a dilution series (below). Mix each tube thoroughly before the next transfer. The undiluted Mouse IL-23 Standard (1000 pg/mL) serves as the high standard. The Calibrator Diluent RD5-3 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, control and standards be assayed in duplicate.

1. Prepare all reagents, working standards, control and samples 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 RD1-14 to each well. *Assay Diluent RD1-14 may contain undissolved material even when mixed well before and during use.*
4. Add 50 µL of standard, control and prepared sample per well. Cover with the adhesive strip provided. **Incubate for 2 hours at room temperature on a horizontal orbital microplate shaker (0.12" orbit) set at 500 ± 50 rpm.** A plate layout is provided for a record of standards and samples assayed.
5. Aspirate each well and wash, repeating the process four times for a total of five 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 100 µL of Mouse IL-23 Conjugate to each well. Cover with a new adhesive strip. **Incubate for 2 hours at room temperature on a horizontal orbital microplate shaker (0.12" orbit) set at 500 ± 50 rpm.**
7. Repeat the aspiration/wash as in step 5.
8. Add 100 µL of TMB Substrate to each well. **Incubate for 30 minutes at room temperature on the benchtop. 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, control 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 mouse IL-23 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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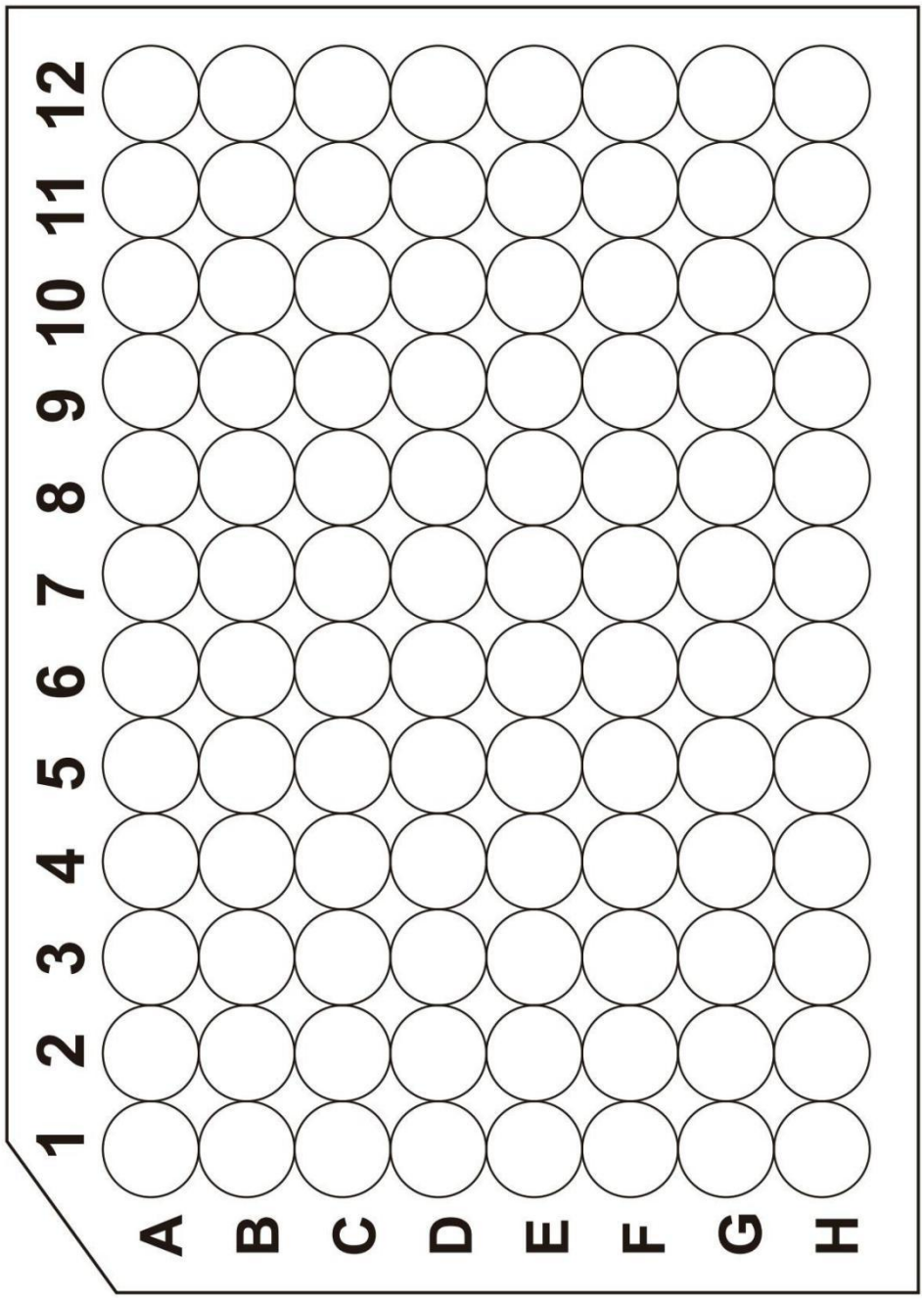
PLATE LAYOUT

Use this plate layout to record standards and samples assayed.



产品信息及操作手册

小鼠 IL-23 Valukine™ ELISA 试剂盒

目录号：VAL637

适用于定量检测天然和重组小鼠白细胞介素 23 (IL-23)的浓度

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目录

I. 背景

白细胞介素 23（Interleukin 23, IL-23）是一种与 IL-12 有关的异源二聚体细胞因子（1-3）。它由两个二硫键连接的亚基组成，一个是 IL-23 独有的 19 kDa（p19）亚基，另一个是与 IL-12 共有的 40 kDa（p40, IL-12β）亚基（3-7）。小鼠 p19 cDNA 编码一个 196 氨基酸（amino acid，aa）的前体，其中有 21 aa 的假定信号肽和 175 aa 的成熟蛋白，其结构与 IL-6 家族的单链细胞因子和 IL-12 的 p35 亚基相似。小鼠 p40 cDNA 编码 335 aa 的前体和 22 aa 的信号肽，以及 313 aa 的成熟蛋白，其中包含四个潜在的糖基化位点、一个 C2 型免疫球蛋白结构域和一个纤维粘连蛋白 III 型结构域。成熟的小鼠 p19 和 p40 与相应的大鼠亚基分别有 88%和 92%的 aa 序列一致性。IL-23 由活化的巨噬细胞、小胶质细胞和单核细胞衍生的树突状细胞产生，以应对病原体，包括某些细菌和病毒和/或其成分（3, 6）。极化 T 细胞也表达 p19 亚基，但这些细胞不表达 p40 亚基（6）。

功能性 IL-23 受体复合物由两个受体亚基组成，即 IL-12 受体β1 亚基（IL-12 receptor β1 subunit, IL-12 Rβ1）和 IL-23 特异性受体亚基（IL-23-specific receptor subunit, IL-23 R）（7）。IL-12 Rβ1 是一种 738 aa 的 I 型跨膜糖蛋白，有一个 546 aa 的胞外结构域，其中包含一个 WSXWS 基序和五个纤连蛋白 III 型结构域。IL-23 R 是一种 644 aa 的 I 型跨膜糖蛋白，具有 351 aa 的胞外结构域，包含两个纤连蛋白 III 型结构域。IL-23 受体复合物在小鼠 Th1 和 Th2 细胞、骨髓树突状细胞、IFN-γ-激活的巨噬细胞和 $CD4^+$ $CD45RB^{low}$ 记忆 T 细胞中均有表达（7）。IL-23 启动的信号转导级联与 IL-12 相似，涉及 Jak2、Tyk2、STAT1、STAT3、STAT4 和 STAT5（7）。IL-23 会与 IL-12 Rβ1 结合，但信号转导需要 IL-23 R 亚基的存在（1-3）。

IL-23 和 IL-12 的生物活性既相互重叠，又截然不同。IL-23 免疫途径可诱导中性粒细胞最早被招募到感染部位，而更典型的宿主防御和细胞毒性反应则是由 IL-12 刺激的（4）。IL-12 驱动 Th1 细胞的发育，并诱导 NK 细胞产生 IFN-γ（3）。相反，IL-23 在 T 细胞亚群 Th17 的发育和维持中发挥作用，该亚群的特点是产生 IL-17A、IL-17F、IL-6 和 TNF-α（3, 4, 8）。Th17 细胞的诱导涉及 TGF-β的作用，而它们的存活和扩增似乎依赖于 IL-23（9-11）。IL-23/IL-17 轴是炎症的重要介质。在小鼠模型中，转基因过表达 IL-23 会导致致死性全身炎症反应（12）。IL-23 对 Th17 细胞的影响还可能促进多种自身免疫性疾病模型的发展，包括实验性过敏性脑脊髓炎（EAE）、胶原诱导性关节炎（CIA）、结肠炎和糖尿病（5, 8, 13-17）。IL-23 还可能在与慢性炎症相关的肿瘤生长加速中发挥作用（18）。在人体中，IL-23 在多种免疫功能失调的病症中被发现上调，包括银屑病、克罗恩病和多发性硬化症（19-21）。

II. 概述

A. 检测原理

本实验采用双抗体夹心ELISA法。抗小鼠IL-23 p19亚基抗体包被于微孔板上。样品，质控品和标准品中的小鼠IL-23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-23 p40亚基检测抗体进行孵育。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样本，小鼠血清样本和小鼠血浆样本；
- 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RD5-3稀释后重新检测；
- 检测结果的不同可由多种因素引起，包括实验人员的操作、移液器的使用方式、洗板技术、反应时间或温度、试剂盒的效期等。

III. 优势

A. 精确度

板内精确度（同一板内不同孔间的精确度）

已知浓度的三个样本，在同一板内分别检测20次，以确定板内精确度。

板间精确度（不同板之间的精确度）

已知浓度的三个样本，在不同板间分别检测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)	49.3	107	344	50.4	116	333
标准差	3.72	5.84	22.7	3.69	7.14	18.6
CV%	7.5	5.5	6.6	7.3	6.2	5.6

B. 回收率

在不同类别样本中掺入检测范围内不同水平的小鼠IL-23，测定其回收率。

样本类型	平均回收率%	范围 (%)
细胞培养基 (n=4)	103	99-108
小鼠血清(n=4)	95	90-101
小鼠肝素血浆(n=4)	99	88-117

C. 灵敏度

40次检测结果表明，小鼠IL-23的最低可测剂量（MDD）范围为0.68-4.17 pg/mL。平均MDD为2.28 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

该免疫测定法以R&D Systems生产的高纯度的*Sf* 21表达的重组小鼠IL-23的共价连接p19和p40亚基进行校正。

E. 线性

不同的样本中含有或掺入高浓度的小鼠IL-23，然后用标准品稀释液RD5-3将样本稀释到检测范围内，测定其线性。

稀释倍数		细胞培养样本 (n=4)	小鼠血清(n=4)	小鼠肝素血浆(n=4)
1:2	平均值/期待值（%）	98	104	102
	范围（%）	93-101	102-105	97-106
1:4	平均值/期待值（%）	98	107	109
	范围（%）	93-102	104-109	104-115
1:8	平均值/期待值（%）	94	107	110
	范围（%）	88-98	100-110	105-115
1:16	平均值/期待值（%）	93	108	110
	范围（%）	85-97	106-110	105-116

F. 样本预值

小鼠血清/血浆样本 - 在此测定中对样品进行小鼠IL-23的可检测水平的评估。未检测到小鼠IL-23。

细胞培养上清样本 - 将J774A.1小鼠网状细胞肉瘤巨噬细胞培养在含10%胎牛血清、2 mM L-谷氨酰胺、100 U/mL青霉素、100 µg/mL硫酸链霉素、25 ng/mL脂多糖（LPS）和100 ng/mL重组小鼠GM-CSF的 DMEM培养基中，培养3天。取出等分的细胞培养上清，检测小鼠IL-23的含量，结果为58.6 pg/mL。

G. 特异性

此ELISA法可检测天然及重组小鼠IL-23。

将以下因子用标准品稀释液RD5-3配制成50 ng/mL的浓度来检测与小鼠IL-23的交叉反应。将50 ng/mL的干扰因子掺入中间范围的小鼠IL-23对照品中，来检测对小鼠IL-23的干扰。没有观察到明显的交叉反应或干扰。

Recombinant mouse:	**Recombinant human:**	**Other recombinants:**
IL-12	IL-12	canine IL-12
IL-12 p35	IL-12 p35	porcine IL-12
IL-12 p40 dimer	IL-23	canine IL-12 p40
IL-12 p40 monomer	**Recombinant human:**	
IL-12 Rβ	IL-23	
IL-23 R	IL-23 p19	
	IL-23 R	

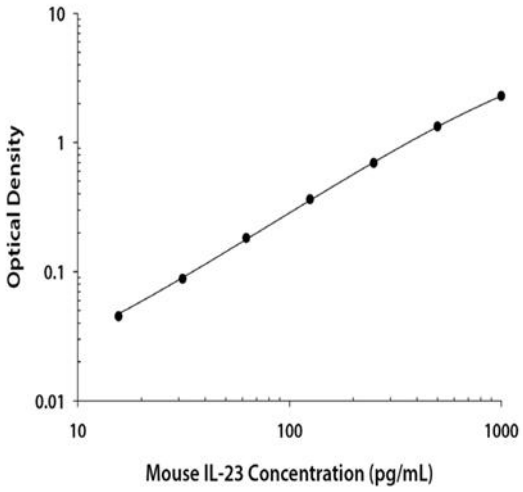
本试剂盒不识别游离的p19或p40亚基。

本试剂盒与重组小鼠 IL-23 p19 不会发生交叉反应，但在浓度≥50 ng/m 时会产生干扰。

IV. 实验

标准曲线实例

该标准曲线数据仅供参考，每次实验应绘制其对应的标准曲线。



(pg/mL)	O.D.	Average	Corrected
0	0.049 0.049	0.049	——
15.6	0.091 0.097	0.094	0.045
31.3	0.137 0.137	0.137	0.088
62.5	0.223 0.238	0.231	0.182
125	0.409 0.415	0.412	0.363
250	0.734 0.749	0.742	0.693
500	1.367 1.389	1.378	1.329
1000	2.299 2.377	2.338	2.289

V. 试剂盒组成及储存

A. 试剂盒组成

组成	描述	规格
Mouse IL-23 Microplate	包被抗小鼠IL-23 p19亚基抗体的96孔聚苯乙烯板，8孔× 12条	1块板
Mouse IL-23 Conjugate	酶标检测抗小鼠IL-23 p40亚基抗体	1瓶
Mouse IL-23 Standard	小鼠IL-23标准品（冻干），参考瓶身标签进行重溶	1瓶
Mouse IL-23 Control	小鼠IL-23质控品（冻干），质控品的测定值应在标签上规定的范围内	1瓶
Assay Diluent RD1-14	检测液（*可能含有沉淀，使用前和使用过程中充分混匀*）	1瓶
Calibrator Diluent RD5-3	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/TMB底物溶液	2瓶
Stop Solution	终止液	1瓶
Plate Sealers	封板膜	3张

B. 试剂盒储存

<table>
<tr><td>未开封试剂盒</td><td colspan="2">2-8℃储存；请在试剂盒有效期内使用</td></tr>
<tr><td rowspan="7">已打开，稀释或重溶的试剂</td><td>洗涤液（1×）</td><td rowspan="6">2-8℃储存，最多30天*</td></tr>
<tr><td>检测液RD1-14</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>标准品稀释液RD5-3</td></tr>
<tr><td>质控品</td><td rowspan="2">2-8℃储存，最多 30 天*</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
- 水平振荡器（0.12”轨道），转速：500±50 rpm
- 用于稀释标准品和样品的聚丙烯管

D. 注意事项

- 试剂盒中的一些组分含有防腐剂，可能引起皮肤过敏反应，避免吸入。
- 试剂盒中的终止液是酸性溶液，使用时请做好眼睛、手、面部及衣服的防护。

VI. 实验前准备

A. 样本收集及储存

以下列出的样品收集和储存条件仅作为一般指南。样本稳定性尚未评估。

细胞培养上清：颗粒物应通过离心去除；立即检测样本或分装，≤ -20 °C储存备用，避免反复冻融。样本可能需要用标准品稀释液RD5-3稀释。

血清样本：血液样本在室温下凝集2小时，然后在2000 × g下离心20分钟。吸取血清样本之后即刻用于检测，或者分装，≤ -20°C储存备用。避免反复冻融。样本可能需要用标准品稀释液RD5-3稀释。

血浆样本：使用肝素作为抗凝剂收集血浆。然后2000 × g离心20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°C储存备用。避免反复冻融。样本可能需要用稀释液RD5-3稀释。

注意： *本试剂盒不推荐使用EDTA血浆样本。*

本试剂盒对枸橼酸钠血浆样本尚未被验证。

本试剂盒不适合使用溶血或黄疸样本。

B. 检测前准备工作

使用前请将所有试剂放置于室温。

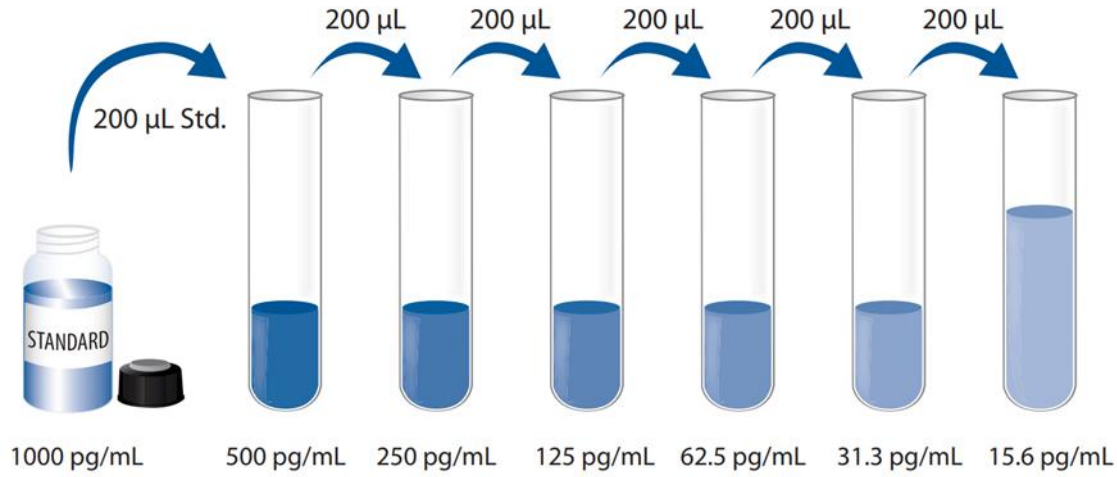
小鼠IL-23质控品：使用1.0 mL去离子水或蒸馏水重溶质控品。混合均匀，测定时不稀释质控品。

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（25×）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×）。

小鼠IL-23标准品：重溶体积请参考瓶身标签*，用标准品稀释液RD5-3重溶小鼠IL-23标准品，请勿使用其他稀释液。得到浓度为1000 pg/mL标准品母液。轻轻震摇至少15分钟，其充分溶解。

*如有疑问，请咨询我们的技术支持。

使用聚丙烯管，向每一稀释管中加入200 µL标准品稀释液RD5-3。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。未稀释的小鼠IL-23标准品可用作标准曲线最高点（1000 pg/mL），标准品稀释液RD5-3可用作标准曲线零点（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，质控品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，标准品，质控品和样本；
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. 每孔加入50μL检测液RD1-14。*检测液RD1-14即使在使用前和使用中已充分混匀，但仍有可能含有沉淀。*
4. 分别将不同浓度标准品，质控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，**在水平振荡器（0.12”轨道）上，转速：500±50 rpm，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小鼠IL-23酶标检测抗体。用封板膜封住反应孔，**在水平振荡器（0.12”轨道）上，转速：500±50 rpm，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，质控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-23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