PRODUCT INFORMATION & MANUAL

Mouse IL-33 Valukine™ ELISA

VAL614

For the quantitative determination of natural and recombinant
Mouse IL-33 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

Version202001.1

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I. BACKGROUND

IL-33, also known as NF-HEV and DVS 27, is a 30 kDa pro-inflammatory protein with intracellular and extracellular activities (1, 2). IL-33 is constitutively expressed in smooth muscle and airway epithelia. It is upregulated by inflammatory stimulation in these cells, keratinocytes, and dermal fibroblasts and by mechanical strain in cardiac fibroblasts (3-5). Mouse IL-33 is synthesized as a 266 amino acid (aa) molecule that contains an N-terminal nuclear localization signal, a helix-turn-helix motif, and a C-terminal region with structural homology to IL-1 family cytokines. Full length IL-33 interacts with nuclear chromatin and functions as a transcriptional repressor (3, 4, 6). Cleavage of full length IL-33 by Caspase-1-like proteases leads to secretion of an 18 kDa C-terminal fragment that is known as mature IL-33 (7-9). Mature mouse IL-33 shares 57% and 90% aa sequence identity with human and rat IL-33, respectively. It shares less than 25% aa sequence identity with other IL-1 family proteins.

Mature IL-33 binds the transmembrane receptor ST2L/IL-1 R4 which subsequently associates with IL-1 R3 (also known as IL-1 RAcP) to enable IL-33 dependent activation of NFκB (7, 10-13). IL-1 R3 is a shared signaling subunit that also associates with IL-1 R1 and IL-1 R6. Alternate splicing of ST2L generates ST2, a soluble decoy receptor that is elevated in the serum of asthma and heart failure patients (5, 14). ST2 association with IL-33 blocks ST2L-dependent signaling and the immunologic and cardiac effects of IL-33 (5, 14).

Secreted IL-33 promotes Th2-biased immune responses, resulting in eosinophilia and allergic inflammation (15). In Th2 cells, it upregulates the production of IL-4, IL-5, and IL-13 as well as ST2L (7, 12). In mast cells, it enhances the production of several cytokines and chemokines but does not trigger degranulation (16-19). It inhibits mast cell apoptosis and functions as a chemoattractant for Th2 cells to sites of inflammation (17, 20). The IL-33/ST2L system has a protective role in the heart. IL-33 interactions with ST2L counteract the cardiac myocyte hypertrophy which is induced by angiotensin II or phenylephrine (5). In parallel with the induction of IL-33 in cardiac fibroblasts, ST2 is induced in cardiac myocytes by mechanical stress (5).

II. OVERVIEW

A. PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for mouse IL-33 has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any mouse IL-33 present is bound by the immobilized antibody. After washing away any unbound substances, an enzyme-linked polyclonal antibody specific for mouse IL-33 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-33 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 Calibrator 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)	45.2	179.3	730.4	47.5	184.7	738.6
Standard Deviation	2.0	4.8	27.4	2.6	6.6	25.7
CV%	4.4	2.7	3.7	5.5	3.6	3.5

B. RECOVERY

The recovery of mouse IL-33 spiked to different levels throughout the range of the assay in cell culture media was evaluated. The recovery ranged from 83.7-107.1% with an average of 98.7%.

The recovery of mouse IL-33 spiked to different levels throughout the range of the assay in mouse serum was evaluated. The recovery ranged from 81.4-98.6% with an average of 89.3%.

C. SENSITIVITY

The minimum detectable dose (MDD) of mouse IL-33 is typically less than 3.6 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 mature IL-33 (aa 109-266) 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-33 and diluted with Calibrator Diluent (1×) to produce samples with values within the dynamic range of the assay.

Dilution	Average % of Expected	Range (%)
1:2	101.5	95.1-110.9
1:4	96.7	91.9-104.8
1:8	93.1	88.3-96.9
1:16	95.2	87.6-105.3

F. SAMPLE VALUES

Serum - Five mouse serum samples were evaluated for the presence of mouse IL-33 in this assay. All samples measured less than the lowest mouse IL-33 standard, 15.6 pg/mL.

G. SPECIFICITY

The following factors prepared at 50 ng/mL were assayed and exhibited no cross-reactivity or interference.

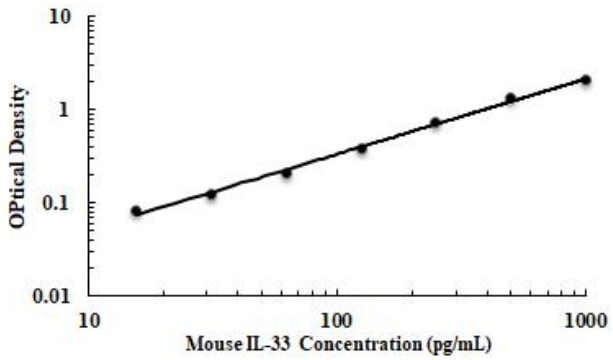
Recombinant mouse
Pro-IL-33 (aa 1-108)
ST2/IL-1 R4/Fc Chimera

A sample containing 6.25 ng/mL of recombinant human IL-33 reads as 105 pg/mL (1.7% cross-reactivity).

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	0.083 0.091	0.087	-
15.6	0.166 0.169	0.168	0.081
31.3	0.207 0.217	0.212	0.125
62.5	0.294 0.299	0.297	0.210
125	0.468 0.475	0.472	0.385
250	0.814 0.815	0.815	0.728
500	1.391 1.403	1.397	1.310
1000	2.160 2.164	2.162	2.075

V. KIT COMPONENTS AND STORAGE

A. MATERIALS PROVIDED

Parts	Description	Size
Mouse IL-33 Microplate	96 well polystyrene microplate (12 strips of 8 wells) coated with a Goat antibody against mouse IL-33.	1 plate
Mouse IL-33 Standard	Recombinant mouse IL-33 in a buffered protein base; lyophilized. Refer to the vial label for reconstitution volume.	2 vials
Mouse IL-33 Detection Antibody	Biotinylated IL-33 polyclonal antibody, lyophilized. Refer to the vial label for reconstitution volume.	1 vial
Calibrator Diluent (4×)	Concentrated buffered diluent used to dilute standard and samples.	1 vial
Streptavidin-HRP B (40×)	40× Streptavidin conjugated to horseradish peroxidase.	1 vial
Reagent Diluent (10×)	A 10× concentrated buffered protein base used to dilute Detection Antibody and HRP.	1 vial
Wash Buffer Concentrate (25×)	A 25× concentrated solution of buffered surfactant with preservatives.	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="9">Opened/ Reconstituted Reagents</td><td>Streptavidin-HRP B</td><td rowspan="4">May be stored for up to 1 month at 2-8 °C.*</td></tr>
<tr><td>Diluted Wash Solution</td></tr>
<tr><td>Unmixed Color Reagent A</td></tr>
<tr><td>Unmixed Color Reagent B</td></tr>
<tr><td>Stop Solution</td><td>Prepare fresh for each assay.</td></tr>
<tr><td>Standard</td><td>Aliquot and store for up to 1 month at -20 °C in a manual defrost freezer. *</td></tr>
<tr><td>Detection antibody</td><td>May be stored for up to 1 month at 2-8 °C.*
Use and discard diluted Reagent Diluent (1×). Prepare fresh for each assay.</td></tr>
<tr><td>Reagent Diluent (10×)</td><td>May be stored for up to 1 month at 2-8 °C.*
Use and discard diluted Calibrator Diluent (1×). Prepare fresh for each assay.</td></tr>
<tr><td>Calibrator Diluent (4×)</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>
<tr><td>Microplate Wells</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

- Some components in this kit contain a preservative which may cause an allergic skin reaction. Avoid breathing mist.
- Color Reagent B may cause skin, eye, and respiratory irritation. Avoid breathing fumes.
- The Stop Solution provided with this kit is an acid solution. Wear protective gloves, clothing, eye, and face protection. Wash hands thoroughly after handling.

VI. PREPARATION

A. SAMPLE COLLECTION AND STORAGE

Cell Culture Supernates - Remove particulates by centrifugation and assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles. Samples may require dilution with Calibrator Diluent (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 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 Buffer - 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.

Calibrator Diluent (1×) - Use deionized or distilled water to prepare Calibrator Diluent (1×).

Reagent Diluent (1×) - Use deionized or distilled water to prepare Reagent Diluent (1×).

Detection Antibody- Reconstitution Volume refer to vial label with Reagent Diluent (1×). Aliquot and store if needed. Dilute stock solution in Reagent Diluent (1×) to the working concentration of 37.5 ng/mL. Prepare at least 15 minutes prior to use.

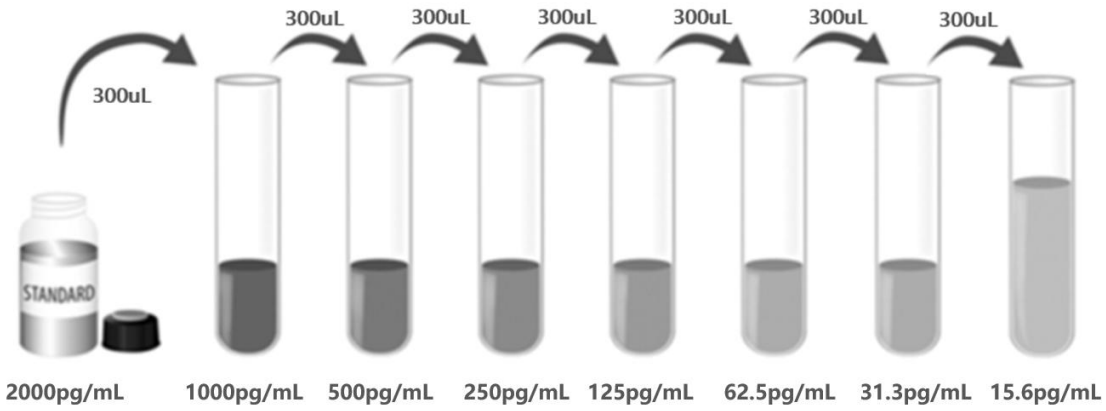
Streptavidin-HRP B (1×) **-** Dilute to the working concentration specified on the vial label using Reagent Diluent (1×).

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.

Mouse IL-33 Standard – Refer to the vial label for the reconstitution volume*. This reconstitution produces a stock solution of 2000 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 300 µL of Calibrator Diluent (1×) into each tube. Use the stock solution to produce a dilution series (below). Mix each tube thoroughly before the next transfer. The 1000 pg/mL standard serves as the high standard. The Calibrator 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, or prepared sample per well. Cover with the adhesive strip provided. Incubate for 2 hours at room temperature. A plate layout is provided for a record of standards and samples assayed. (Samples may require dilution. See Sample Preparation section.)
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 100 µL of the Detection Antibody diluted in Reagent Diluent, to each well. Cover with a new adhesive strip and incubate 2 hours at room temperature.
6. Repeat the aspiration/wash as in step 4.
7. Add 100 µL of the working dilution of Streptavidin-HRP B to each well. Cover the plate and incubate for 30 minutes at room temperature. Avoid placing the plate in direct light.
8. Repeat the aspiration/wash as in step 4.
9. Add 100 µL of Substrate Solution to each well. Incubate for 30 minutes at room temperature. Avoid placing the plate in direct light.
10. Add 50 µL of Stop Solution to each well. Gently tap the plate to ensure thorough mixing.
11. Determine the optical density of each well immediately, 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.

12. 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 IL-33 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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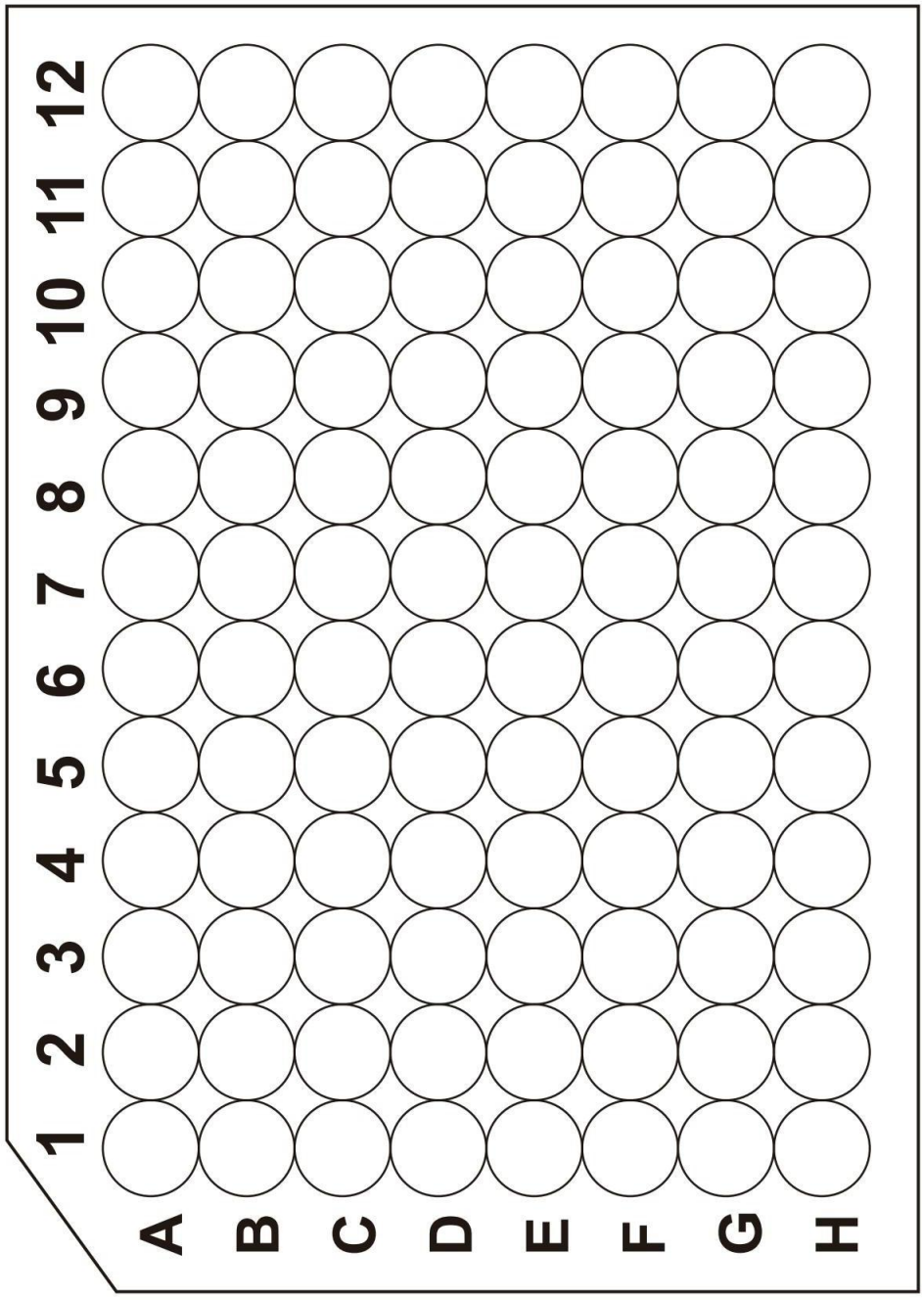
PLATE LAYOUT

Use this plate layout to record standards and samples assayed.



产品信息及操作手册

小鼠 IL-33 Valukine™ ELISA 试剂盒

目录号：VAL614

适用于定量检测天然和重组小鼠 IL-33 的浓度

科研专用，不可用于临床诊断

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目录

I. 背景

IL-33，也被称为NF-HEV和DVS 27，是一种具有细胞内外活性的30 kDa促炎性蛋白 (1, 2)。IL-33在平滑肌和气道上皮组织中为组成性表达。而通过一些细胞，如角质形成细胞和真皮成纤维细胞中的炎症刺激，如心脏成纤维细胞中的机械应变，IL-33可上调其表达 (3-5)。小鼠IL-33由266个氨基酸(aa)分子合成，其中包含一个N-端核定位信号，一个螺旋-转角-螺旋基序，以及一个与IL-1家族细胞因子具有结构同源性的C-端区域。全长IL-33与核染色质相互作用，作为转录抑制因子发挥作用 (3, 4, 6)。Caspase-1样蛋白酶可对全长IL-33进行切割，导致一个18 kDa 的C-末端片段被分泌，即成熟IL-33 (7-9)。成熟小鼠IL-33与人类和大鼠IL-33的aa序列同源性分别为57%和90%。它与其它IL-1家族蛋白的aa序列同源性不到25%。

成熟的IL-33与跨膜受体ST2L/IL-1 R4结合，后者随后与IL-1 R3（也称为IL-1 RAcP）结合，使IL-33依赖性激活NFκB (7, 10-13)。IL-1 R3是一个共享信号亚基，也与IL-1 R1和IL-1 R6相关。ST2L的交替剪接产生ST2，一种可溶性诱饵受体，在哮喘和心力衰竭患者的血清中升高 (5, 14)。ST2与IL-33的结合阻断了ST2L依赖性信号传导，IL-33的免疫和心脏效应 (5, 14)。

分泌的IL-33促进Th2偏向性免疫反应，导致嗜酸性粒细胞增多和过敏性炎症 (15)。在Th2细胞中，它可以上调IL-4、IL-5、IL-13和ST2L (7, 12)。在肥大细胞中，它能促进几种细胞因子和趋化因子的产生，但不会引发脱颗粒 (16-19)。它可以抑制肥大细胞的凋亡，并作为Th2细胞的趋化剂作用于炎症部位 (17, 20)。IL-33/ST2L系统在心脏中起保护作用。IL-33与ST2L的相互作用抵消了血管紧张素II或苯肾上腺素诱导的心肌细胞肥大 (5)。在诱导心肌成纤维细胞产生IL-33的同时，机械应力诱导心肌细胞产生ST2 (5)。

II. 概述

A. 检测原理

本实验采用双抗体夹心 ELISA 法。抗小鼠IL-33单抗包被于微孔板上，样品和标准品中的小鼠IL-33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-33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样本和小鼠血清样本；
- 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)	45.2	179.3	730.4	47.5	184.7	738.6
标准差	2.0	4.8	27.4	2.6	6.6	25.7
CV%	4.4	2.7	3.7	5.5	3.6	3.5

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-33，测定其回收率。回收率范围在 83.7-107.1%， 平均回收率在 98.7%。

在小鼠血清样本中掺入检测范围内不同水平的小鼠 IL-33，测定其回收率。回收率范围在 81.4-98.6%，平均回收率在 89.3%。

C. 灵敏度

小鼠 IL-33 的最低可测剂量（MDD）一般小于 3.6 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

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小鼠成熟IL-33 (aa 109-266) 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-33，然后用标准品稀释剂（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	101.5	95.1-110.9
1:4	96.7	91.9-104.8
1:8	93.1	88.3-96.9
1:16	95.2	87.6-105.3

F. 样本预值

血清样本 - 使用本试剂盒检测了5份小鼠血清样本中 IL-33 的水平。5份样本的检测值均低于IL-33 最低检测限，15.6 pg/mL。

G. 特异性

将以下因子配置成 50 ng/mL的浓度来检测没有观察到明显的交叉反应或干扰。

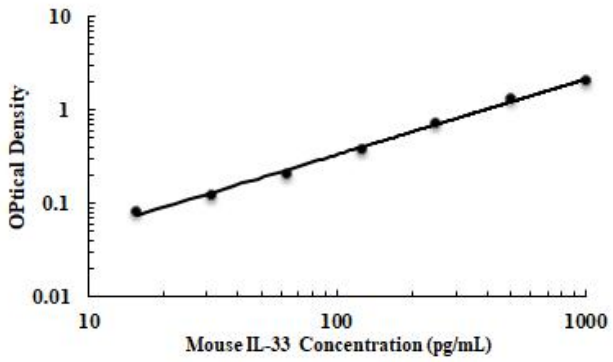
Recombinant mouse
Pro-IL-33 (aa 1-108)
ST2/IL-1 R4/Fc Chimera

样本中含有6.25 ng/mL重组人IL-33 时，检测值为105 pg/mL（1.7%交叉反应）。

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	0.083 0.091	0.087	-
15.6	0.166 0.169	0.168	0.081
31.3	0.207 0.217	0.212	0.125
62.5	0.294 0.299	0.297	0.210
125	0.468 0.475	0.472	0.385
250	0.814 0.815	0.815	0.728
500	1.391 1.403	1.397	1.310
1000	2.160 2.164	2.162	2.075

V. 试剂盒组成及储存

A. 试剂盒组成

组成	描述	规格
Mouse IL-33 Microplate	包被羊抗小鼠 IL-33 抗体的 96 孔聚苯乙烯板，8 孔×12 条	1 块板
Mouse IL-33 Standard	标准品（冻干粉），参考瓶标签进行重溶	2 瓶
Mouse IL-33 Detection antibody	生物素化的 IL-33 检测抗体，冻干粉，参考瓶标签进行重溶	1 瓶
Streptavidin-HRP B (40×)	40×浓缩的链霉亲和素标记的 HRP	1 瓶
Reagent Diluent (10×)	浓缩的试剂稀释液（10×）	1 瓶
Calibrator Diluent (4×)	浓缩的标准品稀释剂（4×）	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>链霉亲和素-HRP B</td><td rowspan="5">2-8℃储存，最多 30 天*</td></tr>
<tr><td>洗涤缓冲液（1×）</td></tr>
<tr><td>显色剂 A</td></tr>
<tr><td>显色剂 B</td></tr>
<tr><td>终止液</td></tr>
<tr><td>标准品</td><td>使用时新鲜配制*</td></tr>
<tr><td>检测抗体</td><td>分装， -20℃储存，最多 30 天*</td></tr>
<tr><td>标准品稀释剂（4×）</td><td>2-8℃储存，最多 30 天*
请每次使用新鲜配制的 1×标准品稀释剂</td></tr>
<tr><td>试剂稀释液（10×）</td><td>2-8℃储存，最多 30 天*
请每次使用新鲜配制的 1×试剂稀释液</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

- 试剂盒中的一些组分含有防腐剂，可能引起皮肤过敏反应，避免吸入。
- Color Reagent B 可能引起皮肤、眼睛和呼吸道刺激，避免吸入。
- 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。使用后请彻底洗手。

VI. 实验前准备

A. 样品收集及储存

细胞培养上清液：颗粒物应离心去除；立刻检测样本。样本收集后若不及时检测，需按一次使用量分装，冻存于-20℃ 冰箱内，避免反复冻融。样本可能需要用标准品稀释剂（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

血清样本需要用标准品稀释剂（1×）2倍稀释后进行检测，例如：100 µL 血清+100 µL 标准品稀释剂（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 ℃。

标准品稀释剂（1×）：使用蒸馏水或去离子水稀释配置成标准品稀释剂（1×）。

试剂稀释液（1×）：使用蒸馏水或去离子水稀释配置成试剂稀释液（1×）。

检测抗体：参考检测抗体瓶标签指示，用试剂稀释液（1×）将冻干粉进行重溶。再用试剂稀释液（1×）稀释至工作浓度37.5 ng/mL，至少在使用前15 分钟准备。

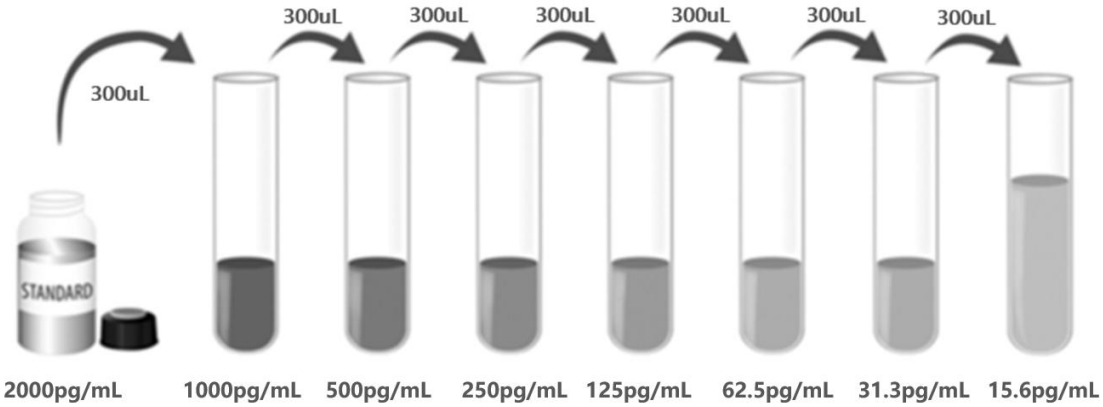
链霉亲和素- HRP B：用试剂稀释液（1×）将链霉亲和素- HRP B（40×）稀释至工作浓度链霉亲和素- HRP B（1×）。

显色剂：按试验所需用量（100 µL/孔）将显色液A和显色液B等体积混合，避光保存，现用现配，须在15分钟内使用。

IL-33 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 2000 pg/mL 标准品母液。轻轻震摇至少 15 分钟，使其充分溶解。

*如有疑问，请咨询我们的技术支持。

各稀释管中加入300 uL标准品稀释剂（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。1000 pg/mL管作标准曲线最高点，标准品稀释剂（1×）可用作标准品零点（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. 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100 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；（血清样本需要稀释，详情参见样本制备部分。）
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400 µL，然后将板内洗涤液吸去。重复操作3次，共洗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100 µL配置好的检测抗体。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100 µL稀释好的链霉亲和素- HRP B 工作液。用封板膜封住反应孔，室温孵育30分钟，**注意避光；**
8. 重复第4步洗板操作；
9. 在每个微孔内加入100 µL显色剂，室温孵育30分钟，**注意避光**；
10. 在每个微孔内加入50 µL终止液，请轻拍微孔板，使溶液混合均匀。孔内溶液颜色会从蓝色变为黄色；
11. 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；
12. **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。通过样本的OD值，可从标准曲线上得到样本中小鼠IL-33的浓度。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