PRODUCT INFORMATION & MANUAL

Human IL-33 Valukine™ ELISA

Catalog Number: VAL176

For the quantitative determination of natural and recombinant
Human 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

Version 202508.2

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I. BACKGROUND

Interleukin-33 (IL-33), also known as NF-HEV and DVS 27, is a 30 kDa pro-inflammatory protein that plays an important role in Th2-biased immune responses and cardiac pathology (1-3). Human IL-33 is synthesized as a 270 amino acid (aa) molecule with an N-terminal nuclear localization signal, a helix-turn-helix motif, and a C-terminal region with structural homology to IL-1 family cytokines (4). Full length IL-33 interacts with nuclear chromatin, binds NFκB, and inhibits pro-inflammatory NFκB transactivation (5-7). Cleavage of full length IL-33 leads to the extracellular release of an 18-20 kDa C-terminal fragment known as mature IL-33 (4, 8). Cathepsin G, Elastase, and Proteinase 3 can each cleave full length IL-33, giving rise to N-terminal heterogeneity of the mature form (9, 10). IL-33 can be inactivated by further cleavage at several sites by Proteinase 3 and Caspase-1 (10, 11). Additional isoforms of human IL-33 with internal deletions are generated by alternative splicing (12, 13). Mature human IL-33 shares 57% and 59% aa sequence identity with mouse and rat IL-33, respectively.

IL-33 binds the transmembrane receptor ST2/IL-1 R4 which subsequently associates with IL-1 RAcP to enable IL-33 dependent signaling (4, 14-16). IL-1 RAcP is a shared signaling subunit that also associates with the receptors IL-1 RI, IL-1 RII, IL-1 R6, and SCF R/c-kit (17). A soluble isoform of ST2 retains the ability to bind IL-33 and blocks ST2-dependent responses (18, 19). Soluble IL-1 RAcP enhances the decoy function of soluble ST2 (16). IL-33 binding to transmembrane ST2 induces the association of ST2 with existing IL-1 RAcP/SCF R complexes (20). Activation of either ST2 or SCF R by their respective ligands can induce signal transduction through the other receptor subunit (20). IL-33 signaling through ST2 additionally triggers VE-Cadherin phosphorylation and internalization on vascular endothelial cells which leads to increased vascular permeability, vessel sprouting, and tubule formation (21).

IL-33 exerts multiple effects on immune system function. It acts on Th2 cells, basophils, and mast cells to induce their migration to sites of inflammation and production of Th2 cytokines (4, 22-26). IL-33 also promotes the expansion of regulatory T cells and alternately activated macrophages while attenuating Th17 cell expansion and activation (27). IL-33 contributes to infection clearance by enhancing neutrophil sensitization to TLR and Dectin-1 signaling, phagocytic activity, and migration to sites of infection (22,

28, 29). It is upregulated in a wide variety of cells under inflammatory conditions (5, 30, 31). Full length IL-33 is also found at elevated levels in bronchiolar lavage fluid during pulmonary fibrosis (32). The full length protein is classified as an alarmin due to its release from physically damaged or necrotic cells and its ability to trigger inflammatory and anti-viral $CD8^+$ T cell responses (11, 33). Like mature IL-33, the full length protein activates ST2 and promotes mast cell activation and neutrophil infiltration (8, 9, 11, 32).

IL-33 induces both protective and pathologic actions in the heart. It counteracts cardiac myocyte hypertrophy and responsiveness to angiotensin II and phenylephrine (18, 34). It is induced in cardiac fibroblasts by mechanical stress and circulates at elevated levels during chronic heart failure (as does the full length form) (18, 35). The soluble ST2 receptor is elevated in the serum of heart failure as well as asthma patients (18, 19). IL-33 inhibits the development of atherosclerotic plaques and induces the production of anti-oxidized LDL antibodies (23). It can also enhance eosinophilic perimyocarditis and impair heart function (34). In other settings, IL-33 limits neutrophil infiltration and circulating inflammatory chemokine levels following hepatic ischemia/reperfusion injury (36) but exacerbates $CD4^+$ T cell infiltration and tissue damage following cisplatin-induced acute kidney injury (37).

II. OVERVIEW

A. PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. An antibody specific for human IL-33 has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any human IL-33 present is bound by the immobilized antibody. After washing away any unbound substances, a biotinylated detection antibody specific for human IL-33 is pipetted into the wells. After washing away any unbound substances, Streptavidin-HRP is pipetted into the wells. Following a wash to remove any unbound reagent, TMB substrate (Chromogenic agent) is added to the wells and color develops in proportion to the amount of human 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 supernates and human 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 (1×) 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)	790.2	197.1	48.2	790.5	196.9	48.3
Standard Deviation	51.6	13.2	3.4	48.9	12.2	3.1
CV%	6.5	6.7	7.0	6.2	6.2	6.4

B. RECOVERY

The recovery of human IL-33 spiked to different levels throughout the range of the assay in cell culture media was evaluated. The recovery ranged from 107.9-119.8% with an average of 112.1%.

The recovery of human IL-33 spiked to different levels throughout the range of the assay in human serum was evaluated. The recovery ranged from 91.2-106.0% with an average of 96.8%.

C. SENSITIVITY

The minimum detectable dose (MDD) of human IL-33 is typically less than 1.595 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 *E. coli* -expressed recombinant human IL-33 produced at R&D Systems.

E. LINEARITY

To assess the linearity of the assay, different samples were containing or spiked with high concentrations of human 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	96.3	89.8-110.8
1:4	94.1	87.5-105.8
1:8	101.2	86.5-113.6
1:16	95.6	80.8-112.5

F. SAMPLE VALUES

Human serum - Six human serum samples were evaluated for the presence of human IL-33 in this assay. All samples measured below the lowest standard, 23.4 pg/mL.

G. SPECIFICITY

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

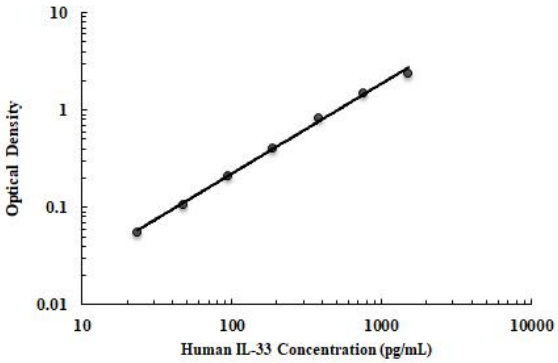
Recombinant human	Recombinant mouse
pro-IL-33 (aa 1-111)	IL-33

Recombinant human ST2/Fc Chimera does not cross react in this assay but does interfere at concentrations > 12.5 ng/mL.

IV. EXPERIMENT

EXAMPLE STANDARD

The standard curve is provided for demonstration only. A standard curve should be generated for each set of samples assayed.



pg/mL	OD	Average	Corrected
0	0.071 0.073	0.072	-
23.4	0.128 0.127	0.128	0.056
46.9	0.179 0.180	0.179	0.107
93.8	0.288 0.284	0.286	0.214
187.5	0.485 0.488	0.487	0.415
375	0.904 0.886	0.895	0.823
750	1.614 1.585	1.599	1.527
1500	2.444 2.485	2.464	2.393

V. KIT COMPONENTS AND STORAGE

A. MATERIALS PROVIDED

Parts	Description	Size
Human IL-33 Microplate	96 well polystyrene microplate (12 strips of 8 wells) coated with an antibody against human IL-33.	1 plate
Human IL-33 Standard	Recombinant human IL-33 in a buffered base; lyophilized. Refer to the vial label for reconstitution volume.	2 vials
Human IL-33 Detection Antibody	Biotinylated human IL-33 antibody, lyophilized. Refer to the vial label for reconstitution volume.	1 vial
Calibrator Diluent Concentrate (2×)	A 2× concentrated buffered diluent used to dilute standard and samples.	1 vial
Reagent Diluent Concentrate (10×)	A 10× concentrated buffered protein base used to dilute Detection Antibody and Streptavidin-HRP A.	1 vial
Streptavidin-HRP A (200×)	200× Streptavidin conjugated to horseradish peroxidase.	1 vial
Wash Buffer Concentrate (25×)	A 25× concentrated solution of buffered surfactant with preservatives.	1 vial
TMB Substrate	TMB ELISA Substrate Solution/TMB Substrate Solution.	1 vial
Stop Solution	2 N sulfuric acid.	1 vial
Plate Sealers	Adhesive strip.	3 strips

B. STORAGE

<table>
<tr><td>Unopened Kit</td><td colspan="2">Store at 2-8 °C. Do not use past kit expiration date.</td></tr>
<tr><td rowspan="7">Opened/ Reconstituted Reagents</td><td>Streptavidin-HRP A</td><td rowspan="4">May be stored for up to 1 month at 2-8 °C.*</td></tr>
<tr><td>Wash Buffer (1×)</td></tr>
<tr><td>TMB Substrate</td></tr>
<tr><td>Stop Solution</td></tr>
<tr><td>Standard</td><td>Prepare fresh for each assay.
Standards may be stored for up to 1 month at -20°C.*</td></tr>
<tr><td>Detection Antibody</td><td>Aliquot and store for up to 1 month at -20 °C in a manual defrost freezer. *</td></tr>
<tr><td>Reagent Diluent Concentrate (10×)</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>Calibrator Diluent Concentrate (2×)</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>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 capable of maintaining a speed of 500 ± 50 rpm.

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 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 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 × g. Remove serum and assay immediately or aliquot and store samples at ≤ -20°C. Avoid repeated freeze-thaw cycles. Samples require dilution with Calibrator Diluent (1×).

B. SAMPLE PREPARATION

Cell culture supernates and human serum samples recommend a 2-fold dilution. A suggested 2-fold dilution is 100 µL of sample + 100 µL of Calibrator Diluent (1×). Optimal dilutions should be determined by the end user.

C. 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×).

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

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

Detection Antibody (1×) - Centrifuge briefly before opening. Reconstitution volume refer to vial label to prepare Detection Antibody (100×). Allow the Detection Antibody to sit for a minimum of 15 minutes with gentle agitation prior to making dilutions. Aliquot and store if needed. Dilute to Detection Antibody (1×) with Reagent Diluent (1×). Prepare at least 15 minutes prior to use.

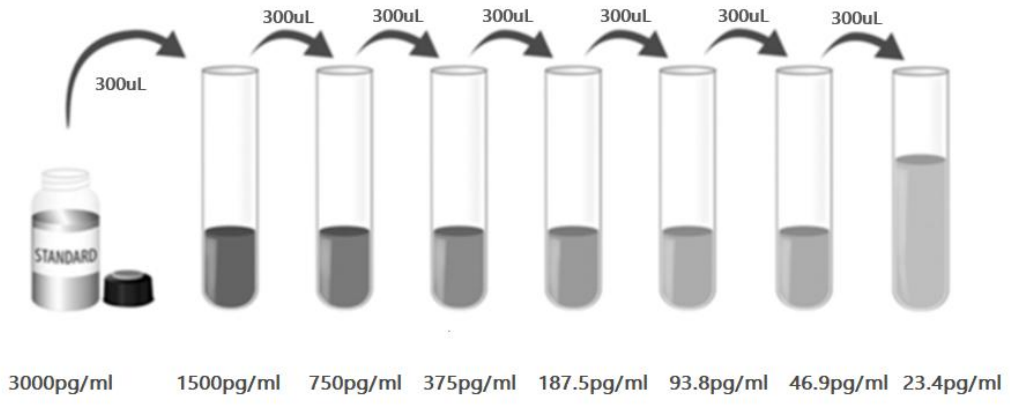
Streptavidin-HRP A (1×) - Centrifuge briefly before opening. Dilute to the working concentration specified on the vial label using Reagent Diluent (1×).

Human IL-33 Standard - Centrifuge briefly before opening. Refer to the vial label

for the reconstitution volume*. This reconstitution produces a stock solution of 3000 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 the appropriate 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 1500 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.
- 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 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 100 µL of standard and prepared sample per well. Cover with the adhesive strip provided. Incubate for **2 hours at room temperature on a horizontal orbital microplate shaker set at 500 ± 50 rpm**. 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 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.
5. Add 100 µL of the Detection Antibody (1×) diluted in Reagent Diluent (1×), to each well. Cover with a new adhesive strip and incubate for **2 hours at room temperature on a horizontal orbital microplate shaker set at 500 ± 50 rpm**.
6. Repeat the aspiration/wash as in step 4.
7. Add 100 µL of the working dilution of Streptavidin-HRP A to each well. Cover the plate and incubate for **30 minutes at room temperature on the benchtop**. **Avoid placing the plate in direct light.**
8. Repeat the aspiration/wash as in step 4.
9. Add 100 µL of TMB Substrate to each well. Incubate for **30 minutes at room temperature on the benchtop**. **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 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.

12. CALCULATION OF RESULTS

Average the duplicate readings for each standard 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 human 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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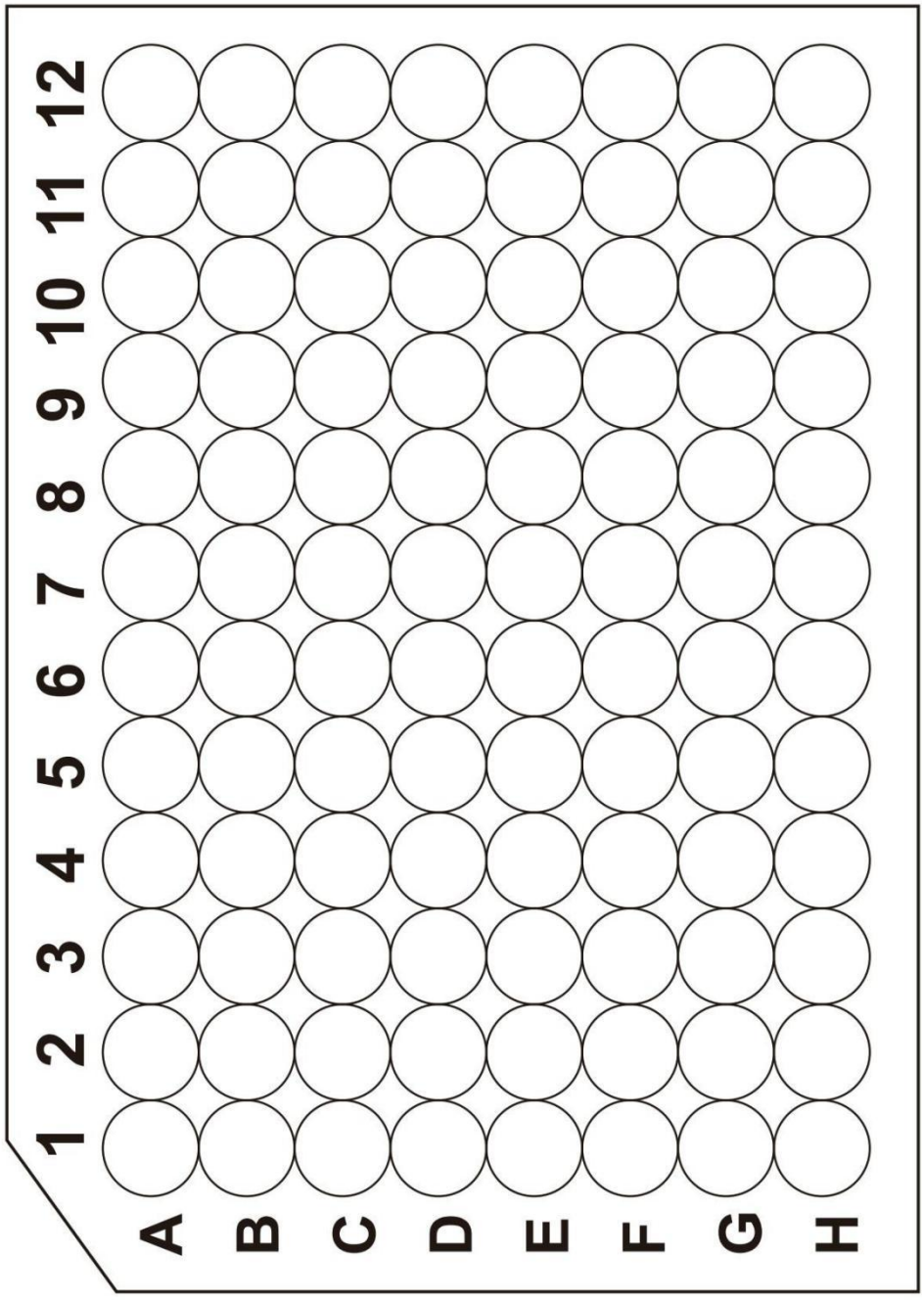
PLATE LAYOUT

Use this plate layout to record standards and samples assayed.



产品信息及操作手册

人 IL-33 Valukine™ ELISA 试剂盒

目录号：VAL176

适用于定量检测天然和重组人 IL-33 的浓度

科研专用，不可用于临床诊断

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有效期详见试剂盒包装标签

Novus 试剂盒确保在你收货日期 3 个月内有效

版本号 202508.2

目录

I. 背景

白细胞介素33 (IL-33)，也称为NF-HEV和DVS 27，是一种30 kDa的促炎蛋白，在Th2偏向的免疫反应和心脏病理学中发挥重要作用(1-3)。人IL-33含有270个氨基酸(aa)分子，具有N端核定位信号、螺旋-转角-螺旋区域以及与IL-1家族细胞因子结构同源的C端区域(4) 。全长IL-33与核染色质相互作用，结合NFκB，并抑制促炎NFκB反式激活(5-7)。全长IL-33的切割导致细胞外释放18-20 kDa的C末端片段，称为成熟IL-33(4, 8)。组织蛋白酶G、弹性蛋白酶和蛋白酶3均可切割全长IL-33，从而导致成熟形式的IL-33(9, 10)。IL-33可通过蛋白酶3和Caspase-1在多个位点进一步切割而失活(10, 11)。通过可变剪接可产生具有内部缺失的其他IL-33异构体(12, 13)。成熟的人IL-33分别与小鼠和大鼠的IL-33具有57%和59%的aa序列一致性。

IL-33结合跨膜受体ST2/IL-1 R4，后者随后与IL-1 RAcP启用依赖IL-33的信号传导(4, 14-16)。IL-1 RAcP是一个共享的信号亚基，也与受体IL-1 RI、IL-1 RII、IL-1 R6和SCF R/c-kit相关联(17)。ST2的可溶性异构体保留结合IL-33的能力并阻断ST2依赖性反应(18, 19)。可溶性IL-1 RAcP增强了可溶性ST2的诱饵功能(16)。IL-33与跨膜ST2结合可诱导ST2与现有的IL-1 RAcP/SCF R复合物结合(20)。ST2或SCF R被它们各自的配体激活可以通过其他受体亚基诱导信号转导(20)。通过ST2发出的IL-33信号还会触发血管内皮细胞上的VE-钙粘蛋白磷酸化和内化，从而导致血管通透性增加、血管发芽和小管形成(21)。

IL-33对免疫系统功能发挥多重作用。它作用于Th2细胞、嗜碱性粒细胞和肥大细胞，以诱导它们迁移到炎症部位并产生Th2细胞因子(4, 22-26)。IL-33还促进调节性T细胞的扩增和交替活化巨噬细胞，同时减弱Th17细胞的扩增和活化(27)。IL-33通过增强中性粒细胞对TLR和Dectin-1信号传导、吞噬活性和迁移到感染部位的敏感性来促进感染清除(22, 28, 29)。在炎症条件下，它在多种细胞中上调(5, 30, 31)。在肺纤维化期间，全长IL-33在细支气管灌洗液中的水平也升高(32)。全长蛋白被归类为警报蛋白，因为它从物理受损或坏死的细胞中释放出来，并且能够触发炎症和抗病毒$CD8^+$ T细胞反应(11, 33)。与成熟的IL-33一样，全长蛋白激活ST2并促进肥大细胞活化和中性粒细胞浸润(8, 9, 11, 32)。

IL-33在心脏中诱导保护作用和病理作用。它可以抵消心脏肌细胞肥大和对血管紧张素II和去氧肾上腺素的反应(18, 34)。它通过机械应力在心脏成纤维细胞中诱导，并在慢性心力衰竭期间以升高的水平循环(全长形式也是如此)(18, 35)。可溶性ST2受体在心力衰竭和哮喘患者的血清中升高(18, 19)。IL-33抑制动脉粥样硬化斑块的发展并诱导抗氧化LDL抗体的产生(23)。它还可以增强嗜酸性心包炎并损害心脏功能(34)。在其他情况下，IL-33会限制肝缺血/再灌注损伤后的中性粒细胞浸润和循环炎症趋化因子水平(36)，但会加剧顺铂诱导的急性肾损伤后的$CD4^+$ T细胞浸润和组织损伤(37)。

II. 概述

A. 检测原理

本实验采用双抗体夹心ELISA法。抗人IL-33抗体包被于微孔板上，样品和标准品中的人IL-33会与固定在板上的抗体结合，游离的成分被洗去；接着加入生物素化的抗人IL-33检测抗体进行孵育，洗涤去除未结合的物质后，加入链霉亲和素标记的辣根过氧化物酶(Streptavidin-HRP)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人血清样本；
- 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液（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)	790.2	197.1	48.2	790.5	196.9	48.3
标准差	51.6	13.2	3.4	48.9	12.2	3.1
CV%	6.5	6.7	7.0	6.2	6.2	6.4

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人IL-33，测定其回收率。回收率范围在107.9-119.8%，平均回收率在112.1%。

在人血清样本中掺入检测范围内不同水平的人IL-33，测定其回收率。回收率范围在91.2-106.0%，平均回收率在96.8%。

C. 灵敏度

人IL-33的最低可测剂量（MDD）一般小于1.595 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*E. coli*表达的高纯度重组人IL-33蛋白所校正。

E. 线性

不同的样本中含有或掺入高浓度的人IL-33，然后用标准品稀释液（1×）将样本稀释到检测范围内，测定其线性。

稀释倍数	平均值 (%)	范围 (%)
1:2	96.3	89.8-110.8
1:4	94.1	87.5-105.8
1:8	101.2	86.5-113.6
1:16	95.6	80.8-112.5

F. 样本预值

人血清样本 - 使用本试剂盒检测了6份人血清样本中人IL-33的水平。所有样本的检测值均低于最低标准品，23.4 pg/mL。

G. 特异性

将以下因子配制成50 ng/mL的浓度来检测没有观察到明显的交叉反应。

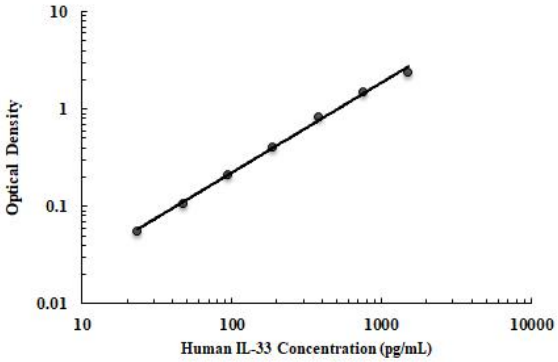
Recombinant human	Recombinant mouse
pro-IL-33 (aa 1-111)	IL-33

重组人ST2/Fc嵌合体在该测定中不发生交叉反应，但在浓度> 12.5 ng/mL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	OD	Average	Corrected
0	0.071 0.073	0.072	-
23.4	0.128 0.127	0.128	0.056
46.9	0.179 0.180	0.179	0.107
93.8	0.288 0.284	0.286	0.214
187.5	0.485 0.488	0.487	0.415
375	0.904 0.886	0.895	0.823
750	1.614 1.585	1.599	1.527
1500	2.444 2.485	2.464	2.393

V. 试剂盒组成及储存

A. 试剂盒组成

组成	描述	规格
Human IL-33 Microplate	包被抗人 IL-33 抗体的 96 孔聚苯乙烯板，8 孔×12 条	1 块板
Human IL-33 Standard	人 IL-33 标准品（冻干粉），参考瓶身标签进行重溶	2 瓶
Human IL-33 Detection Antibody	生物素化的抗人 IL-33 检测抗体，冻干粉，参考瓶身标签进行重溶	1 瓶
Calibrator Diluent Concentrate (2×)	浓缩的标准品稀释液（2×）用于稀释标准品和样本。	1 瓶
Reagent Diluent Concentrate (10×)	浓缩的试剂稀释液（10×）用于稀释检测抗体和 Streptavidin-HRP A。	1 瓶
Streptavidin-HRP A (200×)	200×浓缩的链霉亲和素标记的 HRP	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	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 A</td><td rowspan="4">2-8℃储存，最多 30 天*</td></tr>
<tr><td>洗涤液（1×）</td></tr>
<tr><td>TMB 底物溶液</td></tr>
<tr><td>终止液</td></tr>
<tr><td>标准品</td><td>使用时新鲜配制*
标准品 -20℃储存，最多 30 天*</td></tr>
<tr><td>检测抗体</td><td>分装， -20℃储存，最多 30 天*</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，多余的丢弃</td></tr>
<tr><td>标准品稀释液（2×）</td><td>2-8℃储存，最多 30 天*
请每次使用新鲜配制的 1×标准品稀释液，多余的丢弃</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
- 水平轨道式微孔板振荡器（500 ± 50 rpm）

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。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。样本需要用标准品稀释液（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 × 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。样本需要用标准品稀释液（1×）稀释。

B. 样本准备工作

细胞上清和人血清样本建议用标准品稀释液（1×）2倍稀释后进行检测，例如：100 µL样本+100 µL标准品稀释液（1×）。最佳稀释度应由最终用户确定。

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（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浓缩洗涤液用蒸馏水或去离子水稀释配制成500 mL工作浓度的洗涤液（1×）。

试剂稀释液（1×）：使用去离子水或蒸馏水稀释配制成试剂稀释液（1×）。

标准品稀释液（1×）：使用去离子水或蒸馏水稀释配制成标准品稀释液（1×）。

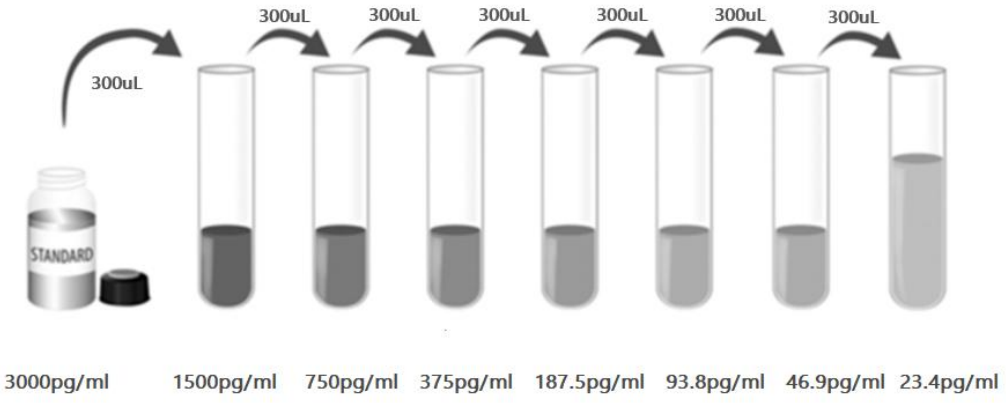
检测抗体（1×）：开盖前请瞬时离心。参考检测抗体瓶标签重溶冻干粉，制备检测抗体（100×）。轻轻震摇至少 15 分钟，其充分溶解。如有需要分装保存。用试剂稀释液（1×）稀释至检测抗体（1×），至少在使用前 15 分钟准备。

链霉亲和素- HRP A（1×）：开盖前请瞬时离心。用试剂稀释液（1×）将链霉亲和素- HRP A（200×）稀释至工作浓度。

人IL-33标准品：开盖前请瞬时离心。冻干标准品的重溶体积请参考瓶身标签，得到浓度为3000 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。

向各稀释管中加入300 µL标准品稀释液（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。1500 pg/mL管作标准曲线最高点，标准品稀释液（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. 分别将不同浓度标准品和实验样本加入相应孔中，每孔100 µL。用封板膜封住反应孔，**室温500 ± 50rpm水平振荡孵育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；（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配制好的检测抗体（1×）。用封板膜封住反应孔，**室温500 ± 50 rpm水平振荡孵育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 A工作液。用封板膜封住反应孔，**静置室温孵育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 TMB底物溶液，**静置室温孵育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；
11. 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；
12. **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。数据可以通过绘制人IL-33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