PRODUCT INFORMATION & MANUAL

Human ST2/IL-33 R Valukine™ ELISA

VAL114

For the quantitative determination of natural and recombinant human ST2 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 202209.3

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I. BACKGROUND

ST2, also known as IL-33 R and T1, is an Interleukin-1 receptor family glycoprotein that contributes to Th2 immune responses (1, 2). ST2 is expressed on the surface of mast cells, activated Th2 cells, macrophages, and cardiac myocytes (3-8). Human ST2 consists of a 310 amino acid (aa) extracellular domain (ECD) with three Ig-like domains, a 21 aa transmembrane segment, and a 207 aa cytoplasmic domain with an intracellular Toll/Interleukin-1 Receptor (TIR) domain (9, 10). Alternate splicing of the 120 kDa human ST2 generates a soluble 60 kDa isoform that lacks the transmembrane and cytoplasmic regions as well as an isoform that additionally lacks the third Ig-like domain (10). Within the ECD, human ST2 shares 68% and 64% aa sequence identity with mouse and rat ST2, respectively.

ST2 binds IL-33, a pro-inflammatory IL-1 family cytokine with intracellular and extracellular activities. IL-33 is constitutively expressed in smooth muscle and airway epithelia (3). It is upregulated by inflammatory stimulation in these cells, keratinocytes, and dermal fibroblasts and by mechanical strain in cardiac fibroblasts (3, 11). Like IL-1, the N-terminal propeptide of IL-33 is cleaved intracellularly to release the C-terminal fragment which is exported as the active cytokine (3, 12). IL-33 binding induces the association of transmembrane ST2 with IL-1 R AcP, a shared signaling subunit that also associates with IL-1 RI and IL-1 Rrp2/IL-1 R6 (13, 14). Soluble ST2 also binds IL-33 and functions as a decoy receptor that blocks the ability of IL-33 to signal through transmembrane ST2 (11, 13, 15-17).

Secreted IL-33 promotes Th2-biased immune responses, resulting in eosinophilia and allergic inflammation (18). It induces the upregulation of inflammatory cytokines and chemokines in Th2 cells and mast cells (3, 19, 20). It also functions as a chemoattractant for Th2 cells to sites of inflammation (21).

In addition to its role in promoting mast cell and Th2 dependent inflammation, transmembrane ST2 activation enhances inflammation-associated hypernociception and protects from atherosclerosis and cardiac myocyte hypertrophy (11, 15, 16). The soluble ST2 isoform is elevated in the serum under inflammatory conditions including allergic asthma, sepsis, trauma, dengue fever, and pulmonary disease (17, 22-25). Serum ST2 elevation is also associated with multiple aspects of heart failure including aortic stenosis, congestive cardiomyopathy, and risk of cardiovascular heart failure and death (26-31).

II. OVERVIEW

A. PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for ST2 has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any ST2 present is bound by the immobilized antibody. After washing away any unbound substances, an enzyme-linked polyclonal antibody specific for ST2 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 ST2 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.**
- The kit is suitable for cell culture supernate and serum.
- The kit should not be used beyond the expiration date on the kit label.
- Do not mix or substitute reagents with those from other lots or sources.
- If samples generate values higher than the highest standard, dilute the samples with Diluent and repeat the assay.
- Any variation in operator, pipetting technique, washing technique, incubation time or temperature, and kit age can cause variation in binding.

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. Assay were performed by at least three technicians using two lots of components.

	Intra-assay Precision			Inter-assay Precision		
Sample	1	2	3	1	2	3
n	20	20	20	40	40	40
Mean (pg/mL)	273	628	1027	262	642	1064
Standard Deviation	15.2	27.5	46.3	18.7	34.7	67.2
CV%	5.6	4.4	4.5	7.1	5.4	6.3

B. RECOVERY

The recovery of human ST2 spiked to different levels throughout the range of the assay in cell culture media was evaluated. The recovery ranged from 89-106% with an average of 97%.

C. SENSITIVITY

The minimum detectable dose (MDD) of ST2 is 2.45-13.5 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 *NS0*-expressed recombinant human ST2 produced at R&D Systems®.

E. LINEARITY

To assess the linearity of the assay, different samples were containing or spiked with high concentrations of ST2 and diluted with Calibrator Diluent 1x to produce samples with values within the dynamic range of the assay.

Dilution	Average % of Expected	Range (%)
1:2	105	102 – 109
1:4	105	102 – 112
1:8	102	90 – 111
1:16	98	85 – 113

F. SAMPLE VALUES

Cell Culture Supernates - BJAB human Burkitt's lymphoma cells (1×10^5 cells/mL) were seeded in DMEM supplemented with 10% fetal bovine serum, 2 mM L-glutamine, 100 U/mL penicillin, and 100 µg/mL streptomycin sulfate. An aliquot of the cell culture supernate was removed, assayed for human ST2, measured 126 pg/mL.

BUD-8 human fbroblast cells were seeded in MEM NEAA 90% supplemented with 10% fetal bovine serum, 2 mM L-glutamine, 100 U/mL penicillin, and 100 µg/mL streptomycin sulfate until confluent. An aliquot of the cell culture supernate was removed, assayed for human ST2, and measured 273 pg/mL.

U-87 MG human glioblastoma/astrocytoma cells (2.1×10^5 cells/mL) were seeded in MEM supplemented with 10% fetal bovine serum, 1% sodium pyruvate, and 2 mM L-glutamine until confluent. An aliquot of the cell culture supernate was removed, assayed for human ST2, and measured 63.9 pg/mL.

HUVEC human umbilical vein endothelial cells (1×10^4 cells/mL) were seeded in EGM-2 media until confluent. An aliquot of the cell culture supernate was removed, assayed for human ST2, and measured 29,920 pg/mL.

HUT-78 human cutaneous T cell lymphoma cells (1×10^4 cells/mL) were seeded in RPMI supplemented with 10% fetal bovine serum, 5 µM β-mercaptoethanol, and 10 ng/mL recombinant human IL-2 until confluent. An aliquot of the cell culture supernate was removed, assayed for human ST2, and measured 54.9 pg/mL.

Serum - Four serum samples were evaluated for the presence of ST2 in this assay. All samples measured ranged from 32148 to 35552pg/mL with an average of 34164.5pg/mL.

G. SPECIFICITY

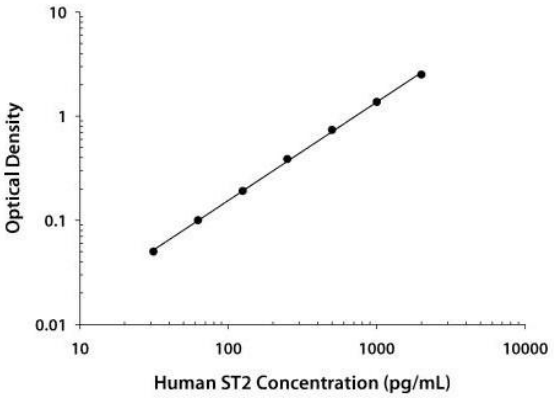
This assay recognizes natural and recombinant human ST2. This assay also recognizes free ST2 and IL-33 complexed ST2. The factors listed below were prepared at 50 ng/mL in Calibrator Diluent 1x and assayed for cross-reactivity. Preparations of the following factors at 50 ng/mL in a mid-range recombinant human ST2 control were assayed for interference. No significant cross-reactivity or interference was observed.

Recombinant Human			**Recombinant Mouse**
IL-1α	IL-1F10	IL-18	IL-33
IL-1β	IL-1rα	IL-18 Rβ	Pro-IL-33
Pro-IL-1β	IL-1 RI	IL-33	ST2
IL-1F5	IL-1 RII	Pro-IL-33	
IL-1F6	IL-1 R AcP	Integrin	
IL-1F7	IL-1 RAPL1	αM/CD11b	
IL-1F8	IL-1 RAPL2	SIGIRR	
IL-1F9	IL-1 Rrp2	Siglec-2/CD22	

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	0.036 0.040	0.038	—
31.3	0.085 0.090	0.088	0.050
62.5	0.137 0.138	0.138	0.100
125	0.228 0.229	0.229	0.191
250	0.420 0.430	0.425	0.387
500	0.764 0.783	0.774	0.736
1000	1.373 1.442	1.408	1.370
2000	2.536 2.552	2.544	2.506

V. KIT COMPONENTS AND STORAGE

A. MATERIALS PROVIDED

Parts	Description	Size
ST2 Microplate	96 well polystyrene microplate (12 strips of 8 wells) coated with a mouse monoclonal antibody against human ST2	1 plate
ST2 Conjugate	solution of polyclonal antibody against ST2 conjugated to horseradish peroxidase with preservatives	1 vial
ST2 Standard	recombinant human ST2; lyophilized	2 vials
Assay Diluent	buffered solution	1 vial
Calibrator Diluent (4×)	a 4x concentrated buffer	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 solution	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>Diluted Wash Solution</td><td rowspan="5">May be stored for up to 1 month at 2-8 °C.*</td></tr>
<tr><td>Stop Solution</td></tr>
<tr><td>Calibrator Diluent 1×</td></tr>
<tr><td>Conjugate</td></tr>
<tr><td>TMB Substrate</td></tr>
<tr><td>Assay diluent</td><td>Use a new standard for each assay. Discard after use.</td></tr>
<tr><td>Standard</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.
- Polypropylene tubes for dilution of standards and samples.

D. PRECAUTION

- 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

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 20-fold dilution. A suggested 20-fold dilution is 10 µL of sample + 190 µL of Calibrator Diluent (1×).

C. REAGENT PREPARATION

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

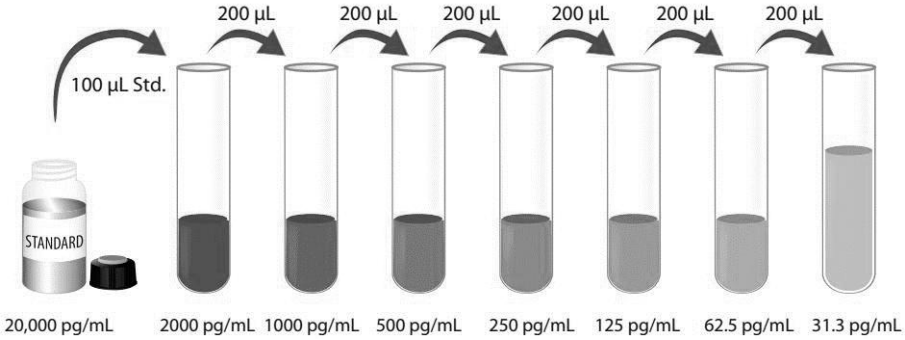
Wash Solution - If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. Dilute 20 mL of Wash Buffer Concentrate (25×) into deionized or distilled water to prepare 500 mL of Wash Buffer.

Calibrator Diluent 1× - Add 20 mL of Calibrator Diluent 4× into 60 mL of deionized or distilled water to prepare 80 mL of Calibrator Diluent 1×.

ST2 Standard- Refer to the vial label for reconstitution volume*. This reconstitution produces a stock solution of 20,000 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 900 µL of Calibrator Diluent 1x into the 2000 pg/mL tube. Pipette 200 µL of Calibrator Diluent 1x into the remaining tube. Use the stock solution to produce a dilution series (below). Mix each tube thoroughly before the next transfer. The 2000 pg/mL standard serves as the high standard. Calibrator Diluent 1x 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 Substrate Solution protected from light. Substrate Solution should change from colorless to gradations of blue.
- Stop Solution should be added to the plate in the same order as the Substrate Solution. The color developed in the wells will turn from blue to yellow upon addition of the Stop Solution. Wells that are green in color indicate that the Stop Solution has not mixed thoroughly with the Substrate Solution.

VII. ASSAY PROCEDURE

Note: **Bring all reagents and samples to room temperature before use. It is recommended that all samples and standards be assayed in duplicate.**

1. Prepare all reagents and working standards as directed in the previous sections.
2. Remove excess microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, and reseal.
3. Add 50 µL of Assay Diluent to each well.
4. Add 50 µL of Standard, sample, or control per well. Cover with the adhesive strip provided. Incubate for 2 hours at room temperature. A plate layout is provided for a record of standards and samples assayed.
5. Aspirate each well and wash, repeating the process three times for a total of four washes. Wash by filling each well with Wash Solution (400 µL) using a squirt bottle, manifold dispenser, or autowasher. Complete removal of liquid at each step is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against clean paper towels.
6. Add 100 µL of ST2 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. The color in the wells should change from blue to yellow. If the color in the wells is green or if the color change does not appear uniform, gently tap the plate to ensure thorough mixing.
10. Determine the optical density of each well within 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. 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 ST2 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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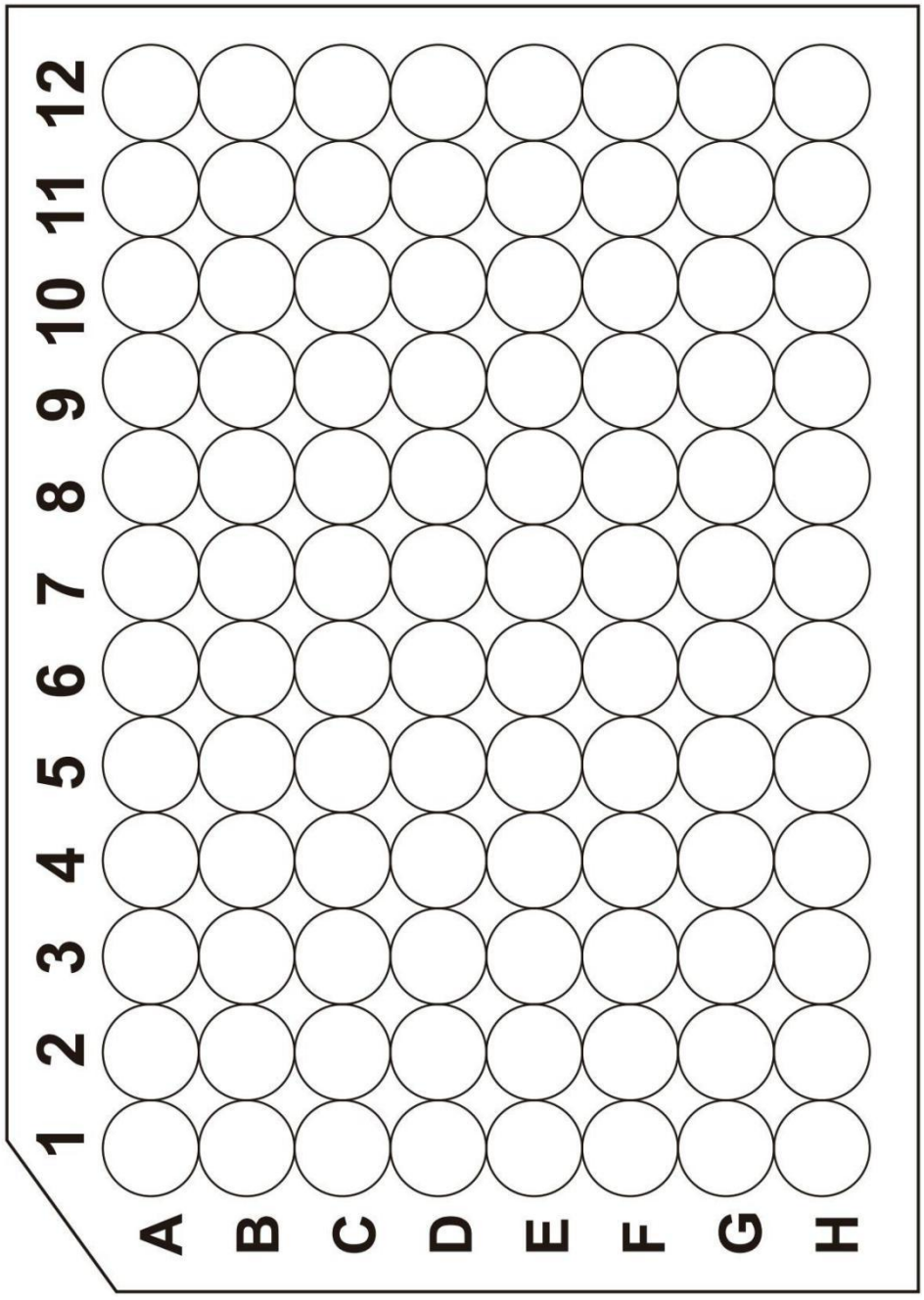
PLATE LAYOUT

Use this plate layout to record standards and samples assayed.



产品信息及操作手册

人 ST2/IL-33R Valukine™ ELISA 试剂盒

目录号：VAL114

适用于定量检测天然和重组人 ST2 的含量

科研专用，不可用于临床诊断

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目录

I. 背景

ST2，也称为IL-33受体和T1，是一种属于白细胞介素-1受体家族的糖蛋白，参与Th2免疫应答（1, 2）。ST2在肥大细胞、活化的Th2细胞、巨噬细胞和心肌细胞（3-8）的表达。人ST2由含有三个Ig样结构域的310个氨基酸（aa）的胞外结构域（ECD）、一个21个氨基酸的跨膜区段和一个含有胞内Toll/白介素-1受体（TIR）结构域（9, 10）的207个氨基酸的胞质结构域组成。120 kDa大小的人ST2的可变剪接可得到一个缺少跨膜区和胞质区的60 kDa可溶ST2亚型，和一个在此基础上进一步缺失了第三个Ig样结构域的亚型（10）。在ECD内，人ST2与小鼠和大鼠ST2的氨基酸序列分别具有68%和64%的同源性。

ST2结合IL-33，后者是一种在细胞内和细胞外都具有活性的IL-1家族促炎性细胞因子。IL-33在平滑肌和气管上皮细胞（3）中持续表达，在上述细胞及角质细胞和皮肤成纤维细胞中受炎性刺激时，及在心脏成纤维细胞（3, 11）中因机械应力而表达上调。与IL-1相似，IL-33的N-末端前肽在细胞内被切割，释放出C-末端片段，即输出有活性细胞因子（3, 12）。与IL-33的结合诱导了跨膜ST2与IL-1R AcP结合，IL-1R AcP为公共信号亚基，也与IL-1 RI和IL-1 Rrp2/IL-1 R6（13, 14）结合。可溶性ST2也与IL-33结合，作为诱骗受体阻断IL-33通过跨膜ST2（11, 13, 15-17）进行的信号传导。

分泌的IL-33促进Th2优势免疫应答，导致嗜酸性粒细胞增多症和过敏性炎症（18）。它诱导Th2细胞和肥大细胞中炎性细胞因子和趋化因子的上调（3, 19, 20）。它还可以作为一种趋化因子诱导Th2细胞到达炎症部位（21）。

除了其在促进肥大细胞和Th2依赖性炎症中的作用，跨膜ST2的活化增强了炎症相关的超伤害感受，防止动脉粥样硬化和心肌细胞肥大（11, 15, 16）。血清中可溶性ST2亚型在炎症状态下升高，包括过敏性哮喘、脓毒症、外伤、登革热和肺部疾病（17, 22-25）。血清ST2升高也与心力衰竭相关，涉及到主动脉瓣狭窄、充血性心肌病、心力衰竭和死亡的风险（26-31）。

II. 概述

A. 检测原理

本实验采用双抗体夹心ELISA法。抗人ST2单抗包被于微孔板上，样品和标准品中存在的ST2与固定在板上的单抗结合，游离的成分被洗去；加入辣根过氧化酶标记的抗人ST2多抗，未结合的抗体被洗去；加入TMB底物溶液，溶液颜色与结合的ST2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；**
- 本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
数量	20	20	20	40	40	40
平均值 (pg/mL)	273	628	1027	262	642	1064
标准差	15.2	27.5	46.3	18.7	34.7	67.2
CV%	5.6	4.4	4.5	7.1	5.4	6.3

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ST2，测定其回收率。回收率范围在89-106%，平均回收率在97%。

C. 灵敏度

人ST2的最低可测剂量（MDD）一般小于2.45-13.5pg/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*NS0*表达的高纯度重组人ST2蛋白所校正。

E. 线性

不同的样本中含有或掺入高浓度的人ST2，然后用稀释剂（1×）将样本稀释到检测范围内，测定其线性。

稀释倍数	平均值/期待值（%）	范围（%）
1:2	105	102 – 109
1:4	105	102 – 112
1:8	102	90 – 111
1:16	98	85 – 113

F. 样本预值

细胞上清样本-按1×10^{5}细胞/mL 比例接种人Burkitt淋巴瘤细胞系BJAB于DMEM培养基，其中含10%FBS、2mM L-谷氨酰胺、100U/mL青霉素, 100μg/mL链霉素，培养至细胞长满后取上清测定ST2，测定值为126pg/mL.

接种人成纤维细胞系BUD-8于MEM NEAA 90%培养基，其中含10% FBS、2mM L-谷氨酰胺、100U/mL青霉素, 100μg/mL链霉素，培养至细胞长满后取上清测定ST2，测定值为273pg/mL.

按 2.1×10^{5}细胞/mL比例接种人胶质母细胞瘤/星形细胞瘤细胞系U-87于MEM培养基，其中含10% FBS、1%丙氨酸钠、2mM L-谷氨酰胺，培养至细胞长满后取上清测定ST2，测定值为63.9pg/mL.

按 1×10^{4}细胞/mL比例接种人脐带静脉内皮细胞HUVEC于EGM-2培养基，培养至细胞长满后取上清测定ST2，测定值为29920 pg/mL.

按 1×10^{4}细胞/mL比例接种人皮肤T细胞淋巴瘤细胞系HUT-78于RPMI培养基，其中含10% FBS、5 μM β-巯基乙醇、10ng/mL重组人IL-2。培养至细胞长满后取上清测定ST2，测定值为54.9 pg/mL.

血清样本-使用本试剂盒检测了4份人血清样本中ST2的水平。4份样本的检测值在32148 - 35552pg/mL之间，平均值为34164.5pg/mL。

G. 特异性

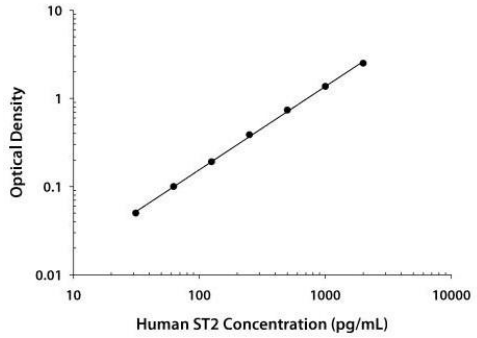
此ELISA法可检测天然及重组人ST2蛋白，也可检测游离ST2以及与IL-33结合的ST2。将以下因子用稀释剂（1×）配置成 50 ng/mL的浓度来检测其与人ST2的交叉反应。将50 ng/mL的干扰因子掺入中间浓度范围的重组人ST2对照品中，来检测其对人ST2的干扰。没有观察到明显的交叉反应或干扰。

重组人蛋白：			**重组小鼠蛋白：**
IL-1α	IL-1F10	IL-18	IL-33
IL-1β	IL-1rα	IL-18 Rβ	Pro-IL-33
Pro-IL-1β	IL-1 RI	IL-33	ST2
IL-1F5	IL-1 RII	Pro-IL-33	
IL-1F6	IL-1 R AcP	Integrin	
IL-1F7	IL-1 RAPL1	αM/CD11b	
IL-1F8	IL-1 RAPL2	SIGIRR	
IL-1F9	IL-1 Rrp2	Siglec-2/CD22	

IV. 实验标准

标准曲线实例

该标准曲线数据仅供参考，每次实验应生成其对应的标准曲线。



(pg/mL)	O.D.	Average	Corrected
0	0.036 0.040	0.038	—
31.3	0.085 0.090	0.088	0.050
62.5	0.137 0.138	0.138	0.100
125	0.228 0.229	0.229	0.191
250	0.420 0.430	0.425	0.387
500	0.764 0.783	0.774	0.736
1000	1.373 1.442	1.408	1.370
2000	2.536 2.552	2.544	2.506

V. 试剂盒组成及储存

A. 试剂盒组成

组成	描述	规格
ST2 Microplate	包被抗体的96孔聚苯乙烯板，8孔×12条	1块板
ST2 Conjugate	酶标检测ST2 抗体	1瓶
ST2 Standard	标准品（冻干）	2瓶
Assay Diluent	检测液	1瓶
Calibrator Diluent（4×）	浓缩稀释剂（4×）	1瓶
Wash Buffer Concentrate（25×）	浓缩洗涤缓冲液（25×）	1瓶
TMB Substrate	TMB底物溶液	1瓶
Stop Solution	终止液	1瓶
Plate Sealers	封板胶纸	3张

B. 试剂盒储存

<table>
<tr><td>未开封试剂盒</td><td colspan="2">2-8℃储存；请在试剂盒有效期内使用</td></tr>
<tr><td rowspan="8">已打开，稀释或重溶的试剂</td><td>洗涤缓冲液（1×）</td><td rowspan="6">2-8℃储存，最多30天*</td></tr>
<tr><td>终止液</td></tr>
<tr><td>稀释剂（1×）</td></tr>
<tr><td>检测液</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</td><td>现配现用；用后即弃。</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；
- 使用聚丙烯试管稀释标准品和样品；
- 洗瓶（喷瓶）、多通道洗板器或自动洗板机；
- 500mL量筒。

D. 注意事项

- 本试剂盒提供的终止液是一种酸性溶液；
- 本试剂盒的某些组份含有会导致皮肤过敏的防腐剂，请避免吸入其挥发气体；
- 穿戴防护手套、衣服、防护眼镜和脸部保护，操作后彻底洗手。

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1000xg离心15分钟。吸取血清样本之后即刻用于检测，或者分装，-20℃贮存备用。避免反复冻融。

B. 样本准备工作

血清样本需要用稀释剂（1×）20倍稀释后进行检测，即10 µL血清+190 µL稀释剂（1×）。

C. 检测前准备工作

使用前请将所有试剂放置于室温

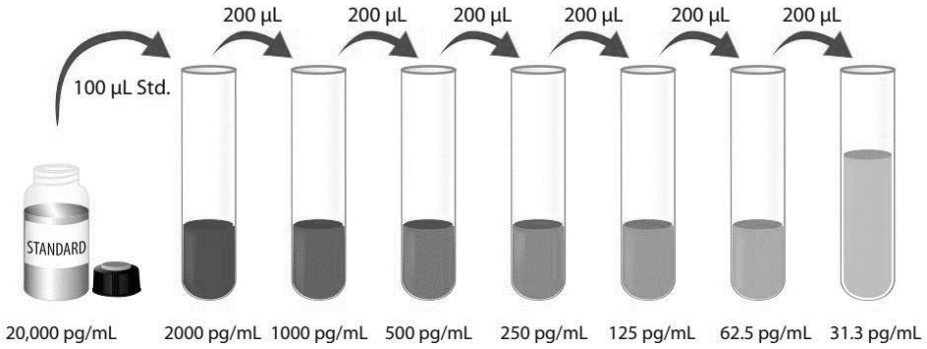
洗涤液：从冰箱中取出的浓缩洗涤液可能有结晶，属于正常现象；放置室温，轻摇混匀，待结晶完全溶解后再配制洗涤液。可将20mL浓缩洗涤液用480mL蒸馏水或去离子水稀释配置成500mL工作浓度的洗涤液。未用完的放回4℃。

稀释剂（1×）：可将20mL浓缩稀释剂（4×）用60mL蒸馏水或去离子水稀释配置成80mL工作浓度的稀释剂。

标准品：依照标准品标签上注明的重溶体积，重溶冻干标准品，得到浓度为20,000pg/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。

使用聚丙烯试管作为标准品稀释管。取900µL稀释剂（1×）至标记浓度为2000pg/mL管中，余下每个稀释管中加入200µL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。2000pg/mL 用作标准曲线最高点，稀释剂（1×）可用作标准曲线零点（0 pg/mL）。



D. 技术小提示

- 当混合或重溶蛋白液时，尽量避免起沫；
- 为了避免交叉污染，配置不同浓度标准品、上样、加不同试剂都需要更换枪头。另外不同试剂请分别使用不同的移液槽；
- 建议15分钟内完成一块板的上样；
- 每次孵育时，正确使用封板胶纸可保证结果的准确性；
- 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. 每孔加入检测液50μL；
4. 分别将不同浓度标准品，实验样本或者质控品加入相应孔中，每孔50μL。用封板胶纸封住反应孔，室温孵育2小时。说明书提供了一张96孔模板图，可用于记录标准品和试验样本的板内位置；
5. 将板内液体吸去，使用洗瓶、多通道洗板器或自动洗板机洗板。每孔加洗涤液400μL，然后将板内洗涤液吸去。重复操作4次。每次洗板尽量吸去残留液体会有助于得到好的实验结果。最后一次洗板结束，请将板内所有液体吸干或将板倒置，在吸水纸拍干所有残留液体；
6. 在每个微孔内加入100μL酶标检测抗体。用封板胶纸封住反应孔，室温孵育2小时；
7. 重复第5步洗板操作；
8. 在每个微孔内加入100μL 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100μL终止液，孔内溶液颜色会从蓝色变为黄色。如果溶液颜色变为绿色或者颜色变化不一致，请轻拍微孔板，使溶液混合均匀；
10. 加入终止液后10分钟内，使用酶标仪测量450nm的吸光度值，设定540nm或570nm作为校正波长。如果没有使用双波长校正，结果准确度可能会受影响；
11. **计算结果：**将每个标准品和样品的校正吸光度值(OD450-OD540/OD570)、复孔读数取平均值，然后减去平均零标准品OD值。使用计算机软件作四参数逻辑(4-PL)曲线拟合创建标准曲线。另一种方法是，可以通过绘制标准品浓度做对数与相应OD值对数生成曲线，并通过回归分析确定最佳拟合线。这个过程可生成一个足够使用但不太精确的数据拟合。若样本经过稀释，计算浓度时应乘以稀释倍数。

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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