PRODUCT INFORMATION & MANUAL

Human Cystatin C Valukine™ ELISA

Catalog Number: VAL250

For the quantitative determination of natural and recombinant human Cystatin C 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

Cystatin C (gene name CST3) is a secreted cysteine protease inhibitor that belongs to the cystatin superfamily (1). It is a protein of 120 amino acids and approximately 13 kDa in its non-glycosylated form; a glycosylated form is reported in rat, but not in mouse (2). Mature human Cystatin C shares 72% amino acid sequence identity with mouse and rat Cystatin C. Cystatin C is susceptible to endoprotease cleavage producing N-terminally truncated forms (1). Cysteine proteases of the papain family, such as Cathepsins B, H, K, L, and S, are the major targets for Cystatin C (3, 4).

Cystatin C is produced in all tissues and is present in all biological fluids, including plasma, urine and cerebrospinal fluid. Cystatin C is freely filtered by the glomeruli. It is then taken up by proximal tubule epithelial cells via megalin-mediated endocytosis, and is metabolized so that it does not return to the bloodstream (1, 5-7). Therefore, Cystatin C serum concentration correlates closely to the glomerular filtration rate (GFR). Its measurement in serum or plasma has been proposed as an indicator of drug nephrotoxicity that is less affected by factors such as gender, age, muscle mass and cirrhosis than creatinine (5, 7, 8). Circulating Cystatin C can, however, be increased during chronic low-level inflammation, in part due to IL-6-mediated increases in Cystatin C production (8). Conversely, the anti-inflammatory cytokines IL-10, IFN-β and IFN-γ can decrease Cystatin C expression and its circulating levels (9-11).

Cystatin C is involved in several disease processes through its regulation of cysteine protease activity (1). In humans, high circulating Cystatin C in the presence of apparently normal kidney function is associated with coronary artery and cardiovascular disease risk (1, 7, 12, 13). In a model of human aortic aneurism, deletion of mouse Cystatin C in ApoE-/- mice promotes inflammation and speeds cathepsin-mediated rupture of the arterial wall *tunica elastica* (14, 15). Circulating Cystatin C has been reported to influence tumor metastasis. Abnormally low Cystatin C levels allow cathepsin B-mediated degradation of extracellular matrix and promote tumor metastasis, while high Cystatin C levels antagonize TGF-β signaling, slowing cancer invasion and growth (16, 17). Cystatin C is an amyloidogenic protein. In humans, the L68Q variant forms dimers and oligomers more easily than the wild type protein under physiological conditions and is the cause for hereditary Cystatin C amyloid angiopathy (3, 18, 19). Cystatin C also inhibits amyloid-β deposition and protects neuronal cells from toxicity in mouse models of Alzheimer's disease (20-22).

II. OVERVIEW

A. PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. An antibody specific for human Cystatin C has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any human Cystatin C present is bound by the immobilized antibody. After washing away any unbound substances, an enzyme-linked antibody specific for human Cystatin C 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 human Cystatin C 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, human serum, human plasma, human saliva, human urine and human milk.
- 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 forty separate assays to assess inter-assay precision.

	Intra-assay Precision			**Inter-assay Precision**		
Sample	1	2	3	1	2	3
Mean (ng/mL)	16.2	29.9	52.6	17.2	30.8	60.9
Standard Deviation	1.07	0.92	2.41	1.21	1.53	3.60
CV%	6.6	3.1	4.6	7.0	5.0	5.9

B. RECOVERY

The recovery of human Cystatin C spiked to levels throughout the range of the assay in various matrices was evaluated.

Sample Type	**Average % Recovery**	**Range (%)**
Cell culture media (n=4)	96	88-102

C. SENSITIVITY

Fifty assays were evaluated and the minimum detectable dose (MDD) of human Cystatin C ranged from 0.030-0.227 ng/mL. The mean MDD was 0.102 ng/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 NS0-expressed recombinant human Cystatin C produced at R&D Systems.

This assay has been correlated to the Cystatin C reference standard supplied by the Joint Research Centre Institute for Reference Materials and Measurements (Catalog # ERM-DA471/ IFCC) with a slope of 1.07 and R^2 value of 0.998.

E. LINEARITY

To assess the linearity of the assay, different samples were containing or spiked with high concentrations of human Cystatin C and diluted with Calibrator Diluent (1×) to produce samples with values within the dynamic range of the assay.

Dilution		Cell culture supernates (n=4)	Human Serum* (n=4)	Human EDTA plasma* (n=4)	Human Heparin plasma* (n=4)	Human Saliva* (n=4)	Human Urine* (n=4)
1:2	Average % of Expected	102	106	105	102	100	106
	Range (%)	95-105	99-111	99-109	97-108	91-107	101-109
1:4	Average % of Expected	101	108	107	107	101	108
	Range (%)	95-108	103-114	100-114	104-109	94-109	105-110
1:8	Average % of Expected	97	105	105	104	97	103
	Range (%)	91-101	100-109	99-111	100-107	87-111	99-105
1:16	Average % of Expected	97	108	105	106	95	96
	Range (%)	88-107	107-109	99-111	98-114	89-112	87-102

*Samples were diluted prior to assay as directed in the Sample Preparation section.

F. SAMPLE VALUES

Human Serum/Plasma/Saliva/Urine - Samples from apparently healthy volunteers were evaluated for the presence of human Cystatin C in this assay. No medical histories were available for the donors used in this study.

Sample Type	Mean (ng/mL)	Range (ng/mL)	Standard Deviation (ng/mL)
Human Serum (n=36)	792	553-1257	161
Human EDTA plasma (n=36)	774	560-1173	155
Human Heparin plasma (n=36)	786	524-1284	177
Human Saliva (n=11)	1259	103-3184	1077
Human Urine (n=12)	62.9	12.6-188	43.8

Cell Culture Supernates:

Human peripheral blood cells (1× 10^6 cells/mL) were cultured in DMEM supplemented with 5% fetal bovine serum, 50 µM β-mercaptoethanol, 2 mM L-glutamine, 100 U/mL penicillin, and 100 µg/mL streptomycin sulfate. Cells were cultured unstimulated or stimulated with 10 µg/mL PHA. Aliquots of the cell culture supernate were removed and assayed for levels of human Cystatin C.

Condition	Day 1 (ng/mL)	Day 5 (ng/mL)
Unstimulated	ND	ND
Stimulated	ND	16

ND=Non-detectable

IMR-90 human lung fibroblast cells were cultured in MEM supplemented with 10% fetal bovine serum, 1.5 g/L sodium bicarbonate, 0.1 mM non-essential amino acids and 0.1 mM sodium pyruvate. An aliquot of the cell culture supernate was assayed for human Cystatin C and measured 23.7 ng/mL.

MCF-7 human breast cancer cells were grown in DMEM/F12 media 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 assayed for human Cystatin C and measured 47.2 ng/mL.

HepG2 hepatocellular carcinoma cells were grown 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 assayed for human Cystatin C and measured 264 ng/mL.

Human Milk - Two human milk samples were assayed for human Cystatin C and measured 1456 ng/mL and 2431 ng/mL, respectively.

G. SPECIFICITY

This assay recognizes natural and recombinant human Cystatin C.

The factors listed below were prepared at 1.0 µg/mL in Calibrator Diluent (1×) and assayed for cross-reactivity. Preparations of the following factors at 1.0 µg/mL in a mid-range recombinant human Cystatin C control were assayed for interference. No significant cross-reactivity or interference was observed.

Recombinant human:	**Recombinant mouse:**
Cathepsin A	Cathepsin A
Cathepsin B	Cathepsin B
Cathepsin C	Cathepsin C
Cathepsin D	Cathepsin C Active
Cathepsin E	Cathepsin D
Cathepsin F	Cathepsin E
Cathepsin L	Cathepsin H
Cathepsin L2 (V)	Cathepsin L
Cathepsin O	Cathepsin L Proform
Cathepsin S	Cathepsin Z
Cathepsin Z	Cathepsin 1
Cystatin A	Cystatin A
Cystatin B	Cystatin B
Cystatin E/M	Cystatin E/M
Cystatin F	Fetuin-A
Cystatin S	HPRG
Cystatin SA	Kininogen

Cystatin SN	
Fetuin-A	
Fetuin-B	
HPRG	
Kininogen	

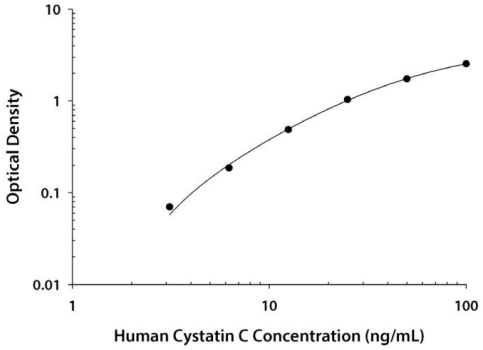
Some cross-reactivity was observed with the following:

Recombinant Factor	**Concentration Tested**	**Cross-reactivity (%)**
Mouse Cystatin C	1.0 µg/mL	0.2
Human Cystatin D	500 ng/mL	0.3

IV. EXPERIMENT

EXAMPLE STANDARD

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



(ng/mL)	O.D.	Average	Corrected
0	0.009 0.010	0.010	—
3.13	0.076 0.082	0.079	0.069
6.25	0.193 0.198	0.196	0.186
12.5	0.491 0.501	0.496	0.486
25	1.027 1.056	1.042	1.032
50	1.699 1.796	1.748	1.738
100	2.504 2.579	2.542	2.532

V. KIT COMPONENTS AND STORAGE

A. MATERIALS PROVIDED

Parts	Description	Size
Human Cystatin C Microplate	96 well polystyrene microplate (12 strips of 8 wells) coated with an antibody against human Cystatin C	1 plate
Human Cystatin C Conjugate	Solution of antibody against human Cystatin C conjugated to horseradish peroxidase	1 vial
Human Cystatin C Standard	Recombinant human Cystatin C in a buffered protein base; lyophilized. Refer to the vial label for reconstitution volume	1 vial
Assay Diluent RD1-43	A buffered protein base	1 vial
Calibrator Diluent Concentrate (5×)/ RD5-24	A 5× concentrated buffered protein base used to dilute standard and samples	1 vial
Wash Buffer Concentrate (25×)	A 25× concentrated solution of buffered surfactant	1 vial
TMB Substrate	TMB ELISA Substrate Solution/TMB Substrate Solution	2 vials
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="8">Opened/ Reconstituted Reagents</td><td>Wash Buffer (1×)</td><td rowspan="4">May be stored for up to 1 month at 2-8°C.*</td></tr>
<tr><td>Stop Solution</td></tr>
<tr><td>Conjugate</td></tr>
<tr><td>TMB Substrate</td></tr>
<tr><td>Standard</td><td>May be stored for up to 1 month at 2-8 °C.*</td></tr>
<tr><td>Assay Diluent RD1-43</td><td>May be stored for up to 1 month at 2-8 °C.*</td></tr>
<tr><td>Calibrator Diluent Concentrate (5×)/ RD5-24</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.
- 100 mL and 500 mL graduated cylinder.
- Collection device for human saliva samples that has no protein binding or filtering capabilities such as a Salivette or equivalent.
- Polypropylene test tubes for dilution.

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.
- Human Cystatin C is detectable in human saliva. Take precautionary measures to prevent contamination of kit reagents while running this assay.

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 (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 may require dilution with Calibrator Diluent (1×).

Plasma - Collect plasma using heparin or EDTA as an anticoagulant. Centrifuge for 15 minutes at 1000 × g within 30 minutes of collection. Assay immediately or aliquot and store samples at ≤ -20 °C. **Do not refreeze aliquots after use.** Samples may require dilution with Calibrator Diluent (1×).

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

Saliva - Collect saliva using a collection device such as a Salivette or equivalent. Assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles. Samples may require dilution with Calibrator Diluent (1×).

Note: *Saliva collector must not have any protein binding or filtering capabilities.*

Urine - Aseptically collect the first urine of the day (mid-stream), voided directly into a sterile container. Centrifuge to remove particulate matter, assay immediately or aliquot and store at ≤ -20 °C. Avoid repeated freeze-thaw cycles. Samples may require dilution with Calibrator Diluent (1×).

Human Milk - Centrifuge for 15 minutes at 1000 × g at 2-8 °C. Collect the aqueous fraction and repeat this process a total of 3 times. Assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles. Samples may require dilution with Calibrator Diluent (1×).

B. SAMPLE PREPARATION

Human serum and plasma samples recommend at least a 30-fold dilution. A suggested 30-fold dilution is 20 µL of sample + 580 µL of Calibrator Diluent (1×). Optimal dilutions should be determined by the end user.

Human saliva samples recommend at least a 20-fold dilution. A suggested 20-fold dilution is 30 µL of sample + 570 µL of Calibrator Diluent (1×). Optimal dilutions should be determined by the end user.

Human milk samples recommend at least a 40-fold dilution. A suggested 40-fold

dilution is 15 µL of sample + 585 µL of Calibrator Diluent (1×). Optimal dilutions should be determined by the end user.

C. REAGENT PREPARATION

The conjugate must remain at 2-8 °C. Bring all reagents to room temperature before use.

Note: *High concentrations of human Cystatin C are found in human saliva. It is recommended that a face mask and gloves be used to protect kit reagents from contamination.*

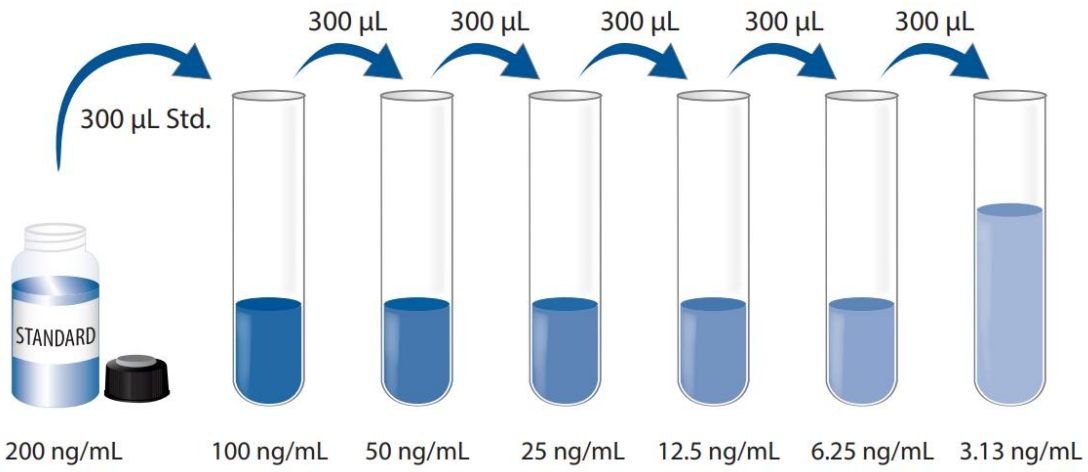
Wash Buffer (1×) - If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. Dilute 20 mL of Wash Buffer Concentrate (25×) into deionized or distilled water to prepare 500 mL of Wash Buffer (1×).

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

Human Cystatin C Standard - Refer to the vial label for the reconstitution volume*. Reconstitute the Human Cystatin C Standard with deionized or distilled water. This reconstitution produces a stock solution of 200 ng/mL. Allow the standard to sit for a minimum of a minimum of 30 minutes with gentle agitation prior to making dilutions.

*If you have any question, please seek help from our Technical Support.

Use polypropylene tubes. 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 100 ng/mL standard serves as the high standard. Calibrator Diluent (1×) serves as the zero standard (0 ng/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 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

The conjugate must remain at 2-8 °C. Bring all other reagents and samples to room temperature before use. It is recommended that all standards and samples be assayed in duplicate.

Note: *High concentrations of human Cystatin C are found in human saliva. It is recommended that a face mask and gloves be used to protect kit reagents from contamination.*

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 Assay Diluent RD1-43 to each well. *Assay Diluent RD1-43 may contain a precipitate. Mix well before and during use.*
4. Add 50 µL of standard and prepared sample per well. Cover with the adhesive strip provided. **Incubate for 3 hours at 2-8 °C.** 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 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 200 µL of **cold** Human Cystatin C Conjugate to each well. Cover with a new adhesive strip. **Incubate for 1 hour at 2-8 °C.**
7. Repeat the aspiration/wash as in step 5.
8. Add 200 µL of TMB Substrate to each well. **Incubate for 30 minutes at room temperature. Protect from light**.
9. Add 50 µL of Stop Solution to each well. Gently tap the plate to ensure thorough mixing.
10. Determine the optical density of each well within 10 minutes, using a microplate reader set to 450 nm. If wavelength correction is available, set to 540 nm or 570 nm. If wavelength correction is not available, subtract readings at 540 nm or 570 nm from the readings at 450 nm. This subtraction will correct for optical imperfections in the plate. Readings made directly at 450 nm without correction may be higher and less accurate.

11. CALCULATION OF RESULTS

Average the duplicate readings for each standard and sample and subtract the average zero standard optical density (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 Cystatin C 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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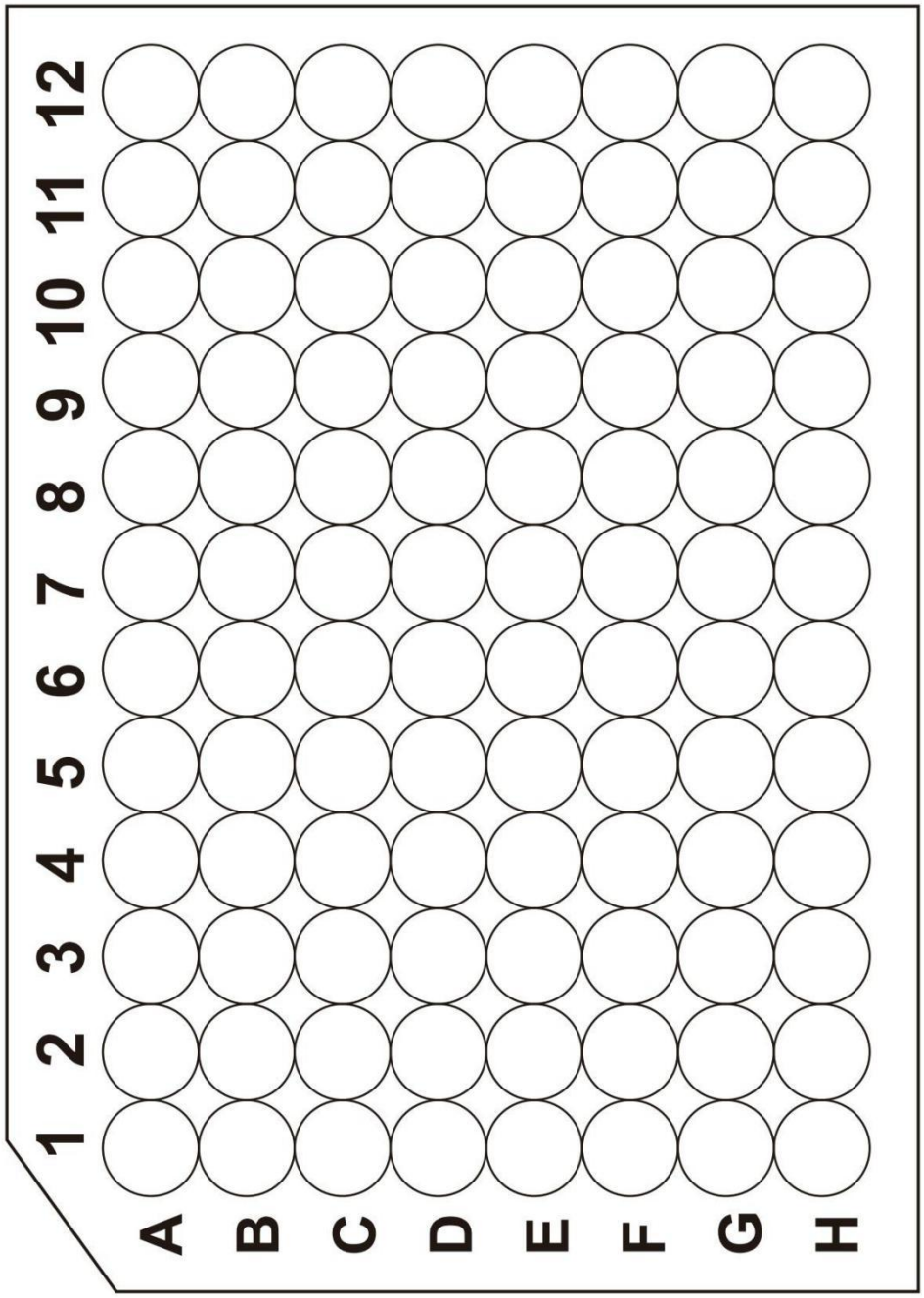
PLATE LAYOUT

Use this plate layout to record standards and samples assayed.



产品信息及操作手册

人 Cystatin C Valukine™ ELISA 试剂盒

目录号：VAL250

适用于定量检测天然和重组人 Cystatin C 的浓度

科研专用，不可用于临床诊断

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版本号 202411.1

目录

I. 背景

胱抑素 C（Cystatin C,基因名称 CST3）是一种分泌型半胱氨酸蛋白酶抑制剂，属于胱抑素超家族（1）。它是一种含有 120 个氨基酸的蛋白质，非糖基化形式的分子量约为 13 kDa；有报告称大鼠体内存在糖基化形式，但小鼠体内没有（2）。成熟的人 Cystatin C 与小鼠和大鼠 Cystatin C 有 72%的氨基酸序列相同。Cystatin C 易被内切蛋白酶裂解，产生 N 端截短形式（1）。木瓜蛋白酶家族的半胱氨酸蛋白酶，如胰蛋白酶 B、H、K、L 和 S，是 Cystatin C 的主要靶标（3, 4）。

Cystatin C 可在所有组织中产生，并存在于所有生物液体中，包括血浆、尿液和脑脊液。Cystatin C 可自由通过肾小球过滤。然后，它通过巨球蛋白介导的内吞作用被近端肾小管上皮细胞吸收，并被代谢掉，从而不会返回血液（1, 5-7）。因此，Cystatin C 血清浓度与肾小球滤过率（glomerular filtration rate, GFR）密切相关。它在血清或血浆中的测量值被认为药物肾毒性的指标，与肌酐相比，它受性别、年龄、肌肉质量和肝硬化等因素的影响较小（5, 7, 8）。然而，在慢性低水平炎症期间，循环中的 Cystatin C 会增加，部分原因是 IL-6 介导的 Cystatin C 生成增加（8）。相反，抗炎细胞因子 IL-10、IFN-β和 IFN-γ可降低 Cystatin C 的表达及其循环水平（9-11）。

Cystatin C 通过调节半胱氨酸蛋白酶的活性参与多种疾病过程（1）。在人体中，肾功能明显正常的情况下，循环中的 Cystatin C 偏高与冠状动脉和心血管疾病风险有关（1, 7, 12, 13）。在人类主动脉瘤模型中，ApoE-/-小鼠体内的小鼠 Cystatin C 基因缺失会促进炎症反应，并加速组织蛋白酶介导的动脉壁弹力膜破裂（14, 15）。据报道，循环中的 Cystatin C 会影响肿瘤转移。Cystatin C 含量异常低会导致由 cathepsin B 介导的细胞外基质降解，促进肿瘤转移，而 Cystatin C 含量高则会拮抗 TGF-β信号传导，减缓癌症的侵袭和生长（16, 17）。Cystatin C 是一种淀粉样蛋白。在人体中，L68Q 变体在生理条件下比野生型蛋白更容易形成二聚体和寡聚体，是遗传 Cystatin C 淀粉样血管病的病因（3，18，19）。Cystatin C 还能抑制淀粉样蛋白-β沉积，保护阿尔茨海默病小鼠模型中的神经细胞免受毒性影响（20-22）。

II. 概述

A. 检测原理

本实验采用双抗体夹心ELISA法。抗人Cystatin C抗体包被于微孔板上，样品和标准品中的人Cystatin C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人Cystatin C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液，人血清、人血浆、人唾液、人尿液和人乳；
- 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×）稀释并重复测定。
- 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（ng/mL）	16.2	29.9	52.6	17.2	30.8	60.9
标准差	1.07	0.92	2.41	1.21	1.53	3.60
CV%	6.6	3.1	4.6	7.0	5.0	5.9

B. 回收率

不同类型样本中掺入检测范围内不同水平的人Cystatin C，测定其回收率。

样本类型	平均回收率（%）	范围（%）
细胞培养基（n=4）	96	88-102

C. 灵敏度

评估 50 次测定，人 Cystatin C 的最小检测剂量（MDD）范围为 0.030-0.227 ng/mL。平均 MDD 为 0.102 ng/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NS0表达的重组人Cystatin C校正。

本测定方法已与由 Joint Research Centre Institute for Reference Materials and Measurements 提供的Cystatin C标准品（目录号 # ERM-DA471/IFCC）校正，斜率为1.07，R^2 值为0.998。

E. 线性

不同的样本中含有或掺入高浓度的人Cystatin C，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		细胞培养上清（n=4）	人血清*（n=4）	人EDTA血浆*（n=4）	人肝素血浆*（n=4）	人唾液*（n=4）	人尿液（n=4）
1:2	平均值/期待值（%）	102	106	105	102	100	106
	范围（%）	95-105	99-111	99-109	97-108	91-107	101-109
1:4	平均值/期待值（%）	101	108	107	107	101	108
	范围（%）	95-108	103-114	100-114	104-109	94-109	105-110
1:8	平均值/期待值（%）	97	105	105	104	97	103
	范围（%）	91-101	100-109	99-111	100-107	87-111	99-105
1:16	平均值/期待值（%）	97	108	105	106	95	96
	范围（%）	88-107	107-109	99-111	98-114	89-112	87-102

*样品在分析前按照样品制备部分的指示进行稀释。

F. 样本预值

人血清/血浆/唾液/尿液 - 在本试验中评估了来自表面健康志愿者的样本中是否存在人 Cystatin C。本研究中使用的供体没有病史。

样本类型	平均值（ng/mL）	范围（ng/mL）	标准差（ng/mL）
人血清（n=36）	792	553-1257	161
人肝素血浆（n=36）	774	560-1173	155
人EDTA血浆（n=36）	786	524-1284	177
人唾液（n=11）	1259	103-3184	1077
人尿液（n=12）	62.9	12.6-188	43.8

细胞培养上清：

人外周血细胞（1×10⁶ cells/mL）在补充5%胎牛血清、50 µM β-巯基乙醇、2 mM L-谷氨酰胺、100 U/mL青霉素和100 µg/mL链霉素硫酸盐的DMEM中培养。细胞在未刺激或用10 µg/mL PHA刺激后培养。取细胞培养上清液，测定人Cystatin C的浓度。

条件	1 天（ng/mL）	5 天（ng/mL）
未刺激	ND	ND
刺激	ND	16

ND=未检出

IMR-90人肺成纤维细胞在含10%胎牛血清、1.5 g/L碳酸氢钠、0.1 mM非必需氨基酸和0.1 mM丙酮酸钠的MEM中培养。细胞培养上清液中人Cystatin C的浓度测定值为23.7 ng/mL。

MCF-7人乳腺癌细胞在补充有10%胎牛血清、2 mML-谷氨酰胺、100 U/mL青霉素和100 µg/mL硫酸链霉素的DMEM/F12培养基中培养。检测细胞培养上清液中人Cystatin C的浓度，测得浓度为47.2 ng/mL。

HepG2肝细胞癌细胞在含有10%胎牛血清、2 mML-谷氨酰胺、100 U/mL青霉素和100 µg/mL链霉素硫酸盐的DMEM中培养。检测细胞培养上清液中人Cystatin C的浓度，测得浓度为264 ng/mL。

人乳 – 对两份人乳样品分别检测人Cystatin C，结果为1456 ng/mL和2431 ng/mL。

G. 特异性

检测方法识别天然和重组人Cystatin C。

以下列出的因子在标准品稀释液（1×）中以1.0 μg/mL的浓度制备，并进行交叉反应性测定。以下列出的因子在中值范围重组人Cystatin C对照品中以1.0 μg/mL的浓度制备，并进行干扰测定。未观察到明显的交叉反应或干扰。

Recombinant human:	**Recombinant mouse:**
Cathepsin A	Cathepsin A
Cathepsin B	Cathepsin B
Cathepsin C	Cathepsin C
Cathepsin D	Cathepsin C Active
Cathepsin E	Cathepsin D
Cathepsin F	Cathepsin E
Cathepsin L	Cathepsin H
Cathepsin L2 (V)	Cathepsin L
Cathepsin O	Cathepsin L Proform
Cathepsin S	Cathepsin Z
Cathepsin Z	Cathepsin 1
Cystatin A	Cystatin A
Cystatin B	Cystatin B
Cystatin E/M	Cystatin E/M
Cystatin F	Fetuin-A
Cystatin S	HPRG
Cystatin SA	Kininogen
Cystatin SN	
Fetuin-A	

Fetuin-B	
HPRG	
Kininogen	

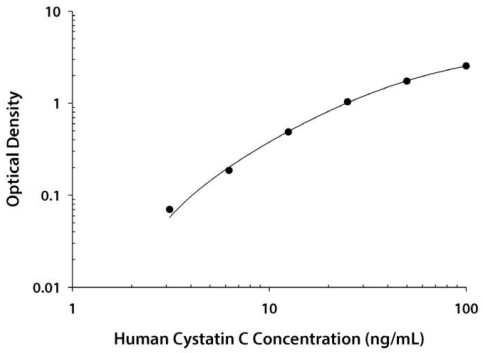
观察到一些交叉反应，如下：

重组因子	浓度测试	交叉反应（%）
Mouse Cystatin C	1.0 µg/mL	0.2
Human Cystatin D	500 ng/mL	0.3

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。



(ng/mL)	O.D.	Average	Corrected
0	0.009 0.010	0.010	—
3.13	0.076 0.082	0.079	0.069
6.25	0.193 0.198	0.196	0.186
12.5	0.491 0.501	0.496	0.486
25	1.027 1.056	1.042	1.032
50	1.699 1.796	1.748	1.738
100	2.504 2.579	2.542	2.532

V. 试剂盒组成及储存

A. 试剂盒组成

组成	描述	规格
Human Cystatin C Microplate	包被抗人Cystatin C抗体的96孔聚苯乙烯板，8孔× 12条	1块板
Human Cystatin C Conjugate	酶标检测抗人Cystatin C抗体	1瓶
Human Cystatin C Standard	重组人Cystatin C标准品（冻干），参考瓶身标签进行重溶	1瓶
Assay Diluent RD1-43	检测液	1瓶
Calibrator Diluent Concentrate (5×)/ RD5-24	浓缩的标准品稀释液（5×），用于稀释标准品和样品	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°C储存；请在试剂盒有效期内使用</td></tr>
<tr><td rowspan="8">已打开，稀释或重溶的试剂</td><td>洗涤液（1×）</td><td rowspan="4">2-8°C储存，最多30天*</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</td><td>2-8°C储存，最多30天*</td></tr>
<tr><td>检测液RD1-43</td><td>2-8°C储存，最多30天*</td></tr>
<tr><td>浓缩的标准品稀释液（5×）/ RD5-24</td><td>2-8°C 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 °C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
- 100 mL和500 mL量筒
- 无蛋白结合或过滤功能的人唾液样品收集装置，例如Salivette或同类产品
- 用于稀释的聚丙烯管

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。
- 人Cystatin C可在人唾液中检测到。在进行此测定时，请采取预防措施，防止试剂盒试剂污染。

VI. 实验前准备

A. 样品收集及储存

以下列出的样品收集和储存条件仅作为一般指南。样品稳定性尚未评估。

细胞培养上清液 - 通过离心去除颗粒物，立即或等分进行检测，并将样品储存在≤ -20°C的温度下，避免反复冻融。样品可能需要用标准品稀释液（1×）稀释。

血清 - 使用血清分离管(SST)，让样本在室温下凝固30分钟，然后在1000 × g的离心力下离心15分钟。分离血清并立即进行检测，或分装并储存在≤ -20 °C。避免反复冻融循环。样本可能需要用标准品稀释液（1×）稀释。

血浆 - 使用肝素或EDTA作为抗凝剂收集血浆。在采样后30分钟内，以1000 × g的离心力离心15分钟。立即检测或分装并储存在≤ -20 °C。**请勿使用后重新冷冻分装**。样品可能需要用标准品稀释液（1×）稀释。

注：*柠檬酸盐血浆在本检测中未经验证。*

唾液 - 使用唾液采集器，如Salivette或等效设备采集唾液。立即检测或分装后于≤-20 °C储存。避免反复冻融循环。样品可能需要用标准品稀释液（1×）进行稀释。

注：*唾液收集器不得具有任何酶结合或过滤功能。*

尿液 - 采集晨尿（中段），直接排入无菌容器。离心去除颗粒物，立即检测或分装并储存在≤ -20 °C。避免反复冻融循环。样品可能需要用标准品稀释液（1×）稀释。

人乳 - 在2-8 °C下，以1000 × g的转速离心15分钟。收集水相，重复此过程共3次。立即测定或分装并储存于≤ -20 °C。避免反复冻融循环。样品可能需要用标准品稀释液（1×）稀释。

B. 样品准备

人血清和血浆样品建议至少稀释 30 倍。建议的 30 倍稀释方式为：将 20 µL 样品加入 580 µL 标准品稀释液（1×）中。最佳稀释倍数应由用户自行确定。

人唾液样品建议至少稀释 20 倍。建议的 20 倍稀释方式为：将 30 µL 样品加入 570 µL 标准品稀释液（1×）中。最佳稀释倍数应由用户自行确定。

人乳样品建议至少稀释 40 倍。建议的 40 倍稀释方式为：将 15 µL 样品加入 585 µL 标准品稀释液（1×）中。最佳稀释倍数应由用户自行确定。

C. 检测前准备工作

酶标检测抗体必须保持在2-8 °C，使用前请将所有试剂放置于室温。

注：*高浓度的人Cystatin C存在于人类唾液中。建议使用口罩和手套以防止试剂盒试剂*

受污染。

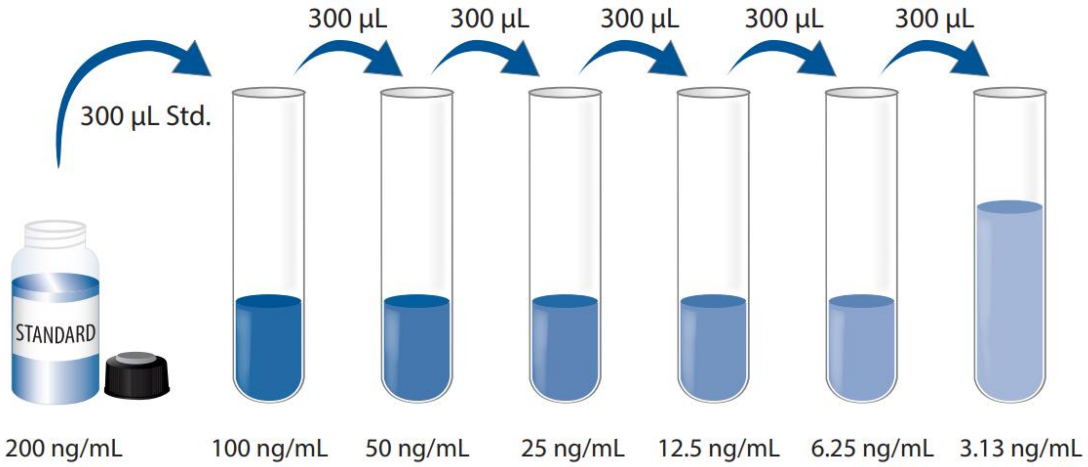
洗涤液（1×）：从冰箱中取出的浓缩洗涤液可能有结晶，属于正常现象；放置室温，轻摇混匀，待结晶完全溶解后再配制洗涤液。可将20 mL浓缩洗涤液（25×）用蒸馏水或去离子水稀释配制成500 mL工作浓度的洗涤液（1×）。

标准品稀释液（1×）：使用去离子水或蒸馏水制备标准品稀释液（1×）。

人Cystatin C标准品：重溶体积请参考瓶身标签*，用去离子水或蒸馏水重构人Cystatin C标准品，得到浓度为200 ng/mL标准品储备母液。轻轻震摇至少30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。用移液管将300 µL标准品稀释液（1×）移入每个管中。使用标准品储备母液稀释（如下）。在每次转移之前，将每个管彻底混合。100 ng/mL作为标准曲线的最高标点。标准品稀释液（1×）作为标准曲线的零点（0 ng/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

酶标检测抗体必须保持在2-8 °C，使用前，将所有其他试剂和样品带至室温。建议对所有标准品和样品进行复孔检测。

注：*高浓度的人Cystatin C存在于人类唾液中。建议使用口罩和手套以防止试剂盒试剂受污染。*

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. 向每个孔中加入100 µL的检测液RD1-43。*检测液RD1-43中可能含有结晶沉淀。使用前和使用过程中请充分混合。*
4. 分别将不同浓度标准品和实验样本加入相应孔中，每孔50 µL。用封板膜封住反应孔，**2-8 °C孵育3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；
6. 在每个微孔内加入200 µL冷的人Cystatin C酶标检测抗体。用新的封板膜封住反应孔，**2-8 °C孵育1小时**；
7. 重复第5步洗板操作；
8. 在每个微孔内加入200 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50 µL终止液，请轻拍微孔板，使溶液混合均匀；
10. 加入终止液后10分钟内，使用酶标仪测量450 nm的吸光度值，设定540 nm或570 nm作为校正波长。如果波长校正不可用，以450 nm的读数减去540 nm或570 nm的读数。这种减法将校正酶标板上的光学缺陷。没有校正而直接在450 nm处进行的读数可能会更高且更不准确；
11. 计算结果：

    将每个标准品和样品的复孔吸光值取平均值，然后减去零标准品平均OD值（O.D.），使用计算机软件作四参数逻辑（4-PL）曲线拟合创建标准曲线。另一替代方法是，通过绘制y轴上每个标准品的平均吸光值与x轴上的浓度来构建标准曲线，并通过图上的点绘制最佳拟合曲线。数据可以通过绘制人Cystatin C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