PRODUCT INFORMATION & MANUAL

Mouse/Rat Cystatin C Valukine™ ELISA

Catalog Number: VAL650

For the quantitative determination of natural and recombinant mouse or rat 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, extracellular cysteine protease inhibitor that belongs to the cystatin superfamily (1-4). It is a protein of 120 amino acids (aa) and approximately 13 kDa in its non-glycosylated form; a glycosylated form is reported in rat, but not in mouse (2-4). Mouse and rat Cystatin C share 72% aa sequence identity with human Cystatin C and 88% aa sequence identity with each other. Cystatin C is susceptible to endoprotease cleavage producing N-terminally truncated forms (3, 4). Cysteine proteases of the papain family, such as Cathepsins B, H, K, L, and S, are the major targets for Cystatin C (5, 6).

Cystatin C is produced in all tissues and is present in all biological fluids. 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, 7-9). 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 (1, 7, 9). Circulating Cystatin C can, however, be increased during chronic low-level inflammation, in part due to IL-6-mediated increases in Cystatin C production (1). Conversely, the anti-inflammatory cytokines IL-10, IFN-β, and IFN-γ can decrease Cystatin C expression and its circulating levels (10-12).

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 an indicator of coronary artery and cardiovascular disease risk (1, 9, 13, 14). 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 (15, 16). 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 (1, 17, 18). Cystatin C is an amyloidogenic protein. In humans, the L68Q variant forms dimers and oligomers more easily than wild type protein under physiological conditions and is the cause for hereditary Cystatin C amyloid angiopathy

(5, 19, 20). Cystatin C also inhibits amyloid-β deposition and protects neuronal cells from toxicity in mouse models of Alzheimer's disease (21-23).

II. OVERVIEW

A. PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. An antibody specific for mouse/rat Cystatin C has been pre-coated onto a microplate. Standards, control and samples are pipetted into the wells and any mouse/rat Cystatin C present is bound by the immobilized antibody. After washing away any unbound substances, an enzyme-linked antibody specific for mouse/rat 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 mouse/rat 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 supernates, tissue lysates, mouse/rat serum, mouse/rat plasma and mouse/rat urine.
- 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)	434	894	3065	465	942	3257
Standard Deviation	14.9	29.2	85.9	43.5	51.2	213
CV%	3.4	3.3	2.8	9.4	5.4	6.5

B. RECOVERY

The recovery of mouse/rat Cystatin C spiked into cell culture media was evaluated.

Sample Type	Average % Recovery	Range (%)
Cell culture media (n=4)	107	98-113

C. SENSITIVITY

Forty-one assays were evaluated and the minimum detectable dose (MDD) of mouse/rat Cystatin C ranged from 2.47-12.9 pg/mL. The mean MDD was 3.93 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 NS0-expressed recombinant mouse Cystatin C 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/rat Cystatin C and diluted with Calibrator Diluent (1×) to produce samples with values within the dynamic range of the assay. Samples were diluted prior to assay.

Mouse Sample		**Cell culture supernates (n=4)**	**Tissue lysates (n=4)**	**Serum (n=4)**	**EDTA plasma (n=4)**	**Heparin plasma (n=4)**	**Urine (n=4)**
1:2	Average % of Expected	98	99	97	97	96	100
	Range (%)	87-101	97-100	96-98	93-100	94-101	99-101
1:4	Average % of Expected	98	97	96	95	95	102
	Range (%)	86-106	94-100	90-100	90-100	89-103	99-107
1:8	Average % of Expected	98	98	96	97	98	104
	Range (%)	88-110	93-103	87-101	92-103	94-108	103-104
1:16	Average % of Expected	99	100	95	101	92	103
	Range (%)	90-111	90-109	89-104	91-110	89-94	98-108

Rat Sample		Cell culture supernates (n=4)	Serum (n=4)	EDTA plasma (n=4)	Heparin plasma (n=4)	Urine (n=4)
1:2	Average % of Expected	92	93	97	91	94
	Range (%)	91-94	81-99	95-98	89-93	90-98
1:4	Average % of Expected	88	91	98	86	90
	Range (%)	84-92	80-100	96-99	83-89	84-95
1:8	Average % of Expected	86	92	98	85	89
	Range (%)	84-88	85-96	95-100	83-86	83-96
1:16	Average % of Expected	80	93	97	85	88
	Range (%)	80-81	87-100	91-101	81-88	83-96

F. SAMPLE VALUES

Mouse/Rat serum/plasma/urine - Mouse and rat samples were evaluated for the presence of mouse and rat Cystatin C in this assay.

Mouse Samples	Mean (ng/mL)	Range (ng/mL)	Standard Deviation (ng/mL)
Serum (n=20)	447	267-638	103
EDTA plasma (n=20)	368	256-515	75.6
Heparin plasma (n=20)	333	254-495	57.6
Urine (n=20)	119	7.45-241	60.4

Rat Samples	Mean (ng/mL)	Range (ng/mL)	Standard Deviation (ng/mL)
Serum (n=20)	2111	1403-2678	360
EDTA plasma (n=20)	1542	963-1972	288
Heparin plasma (n=20)	1535	1002-2054	287
Urine (n=20)	603	239-1383	316

Cell Culture Supernates:

Organs from mice or rats were removed, rinsed in 1× PBS, and kept on ice in 1× PBS. Organs were then cut into 1-2 mm pieces and homogenized using a tissue homogenizer. Cells were seeded into media containing RPMI 1640 supplemented with 10% fetal bovine serum, 2 mM L-glutamine, 100 U/mL penicillin, and 100 µg/mL streptomycin sulfate. Cells were cultured as indicated in the tables below. Aliquots of the cell culture supernates were removed and assayed for levels of mouse/rat Cystatin C.

Mouse Tissue	(ng/mL)
Brain (1 day)	70.1
Heart (3 days)	8.4
Kidney (3 days)	29.0
Liver (3 days)	7.7
Lung (3 days)	12.6
Spleen (3 days)	14.4

Rat Tissue	(ng/mL)
Brain (18 hours)	225
Heart (18 hours)	10.2
Kidney (18 hours)	104
Lung (18 hours)	31.1
Spleen (18 hours)	8.6

3T3-L1 undifferentiated mouse embryonic fibroblast adipose-like cells (2×10^6 cells/T75 flask) were cultured in DMEM supplemented with 10% fetal bovine serum, 2 mM L-glutamine, 100 U/mL penicillin, 100 µg/mL streptomycin sulfate, and incubated for 3 days. The media was removed and 50 mL of fresh media was added and incubated for 4 additional days. An aliquot of the cell culture supernate was removed, assayed for mouse/rat Cystatin C, and measured 166 ng/mL.

3T3-L1 differentiated mouse embryonic fibroblast adipose-like cells (2×10^6 cells/T75 flask) were cultured in DMEM supplemented with 10% fetal bovine serum, 2 mM L-glutamine, 100 U/mL penicillin, 100 µg/mL streptomycin sulfate, and incubated for 3 days. The media was removed and 10% fetal bovine serum, 2 mM L-glutamine, 100 U/mL penicillin, 100 µg/mL streptomycin sulfate, 1 µg/mL bovine insulin, 0.5 mM MIX, and 1 µM DEX was added and incubated for 4 additional days. An aliquot of the cell culture supernate was removed, assayed for mouse/rat Cystatin C, and measured 507 ng/mL.

Tissue Lysates - Organs from mice were rinsed with 1 × PBS and homogenized with a tissue homogenizer in 1 × PBS. An equal volume of Cell Lysis Buffer 2 was added and tissues were lysed at room temperature for 30 minutes with gentle agitation. Debris was then removed by centrifugation. An aliquot of each tissue lysate was removed and assayed for levels of mouse Cystatin C.

Mouse Tissue	(ng/mL)
Brain	1254
Heart	210
Kidney	407
Liver	26.7
Lung	169
Spleen	127

G. SPECIFICITY

This assay recognizes natural and recombinant mouse and rat Cystatin C.

The factors listed below were prepared at 80 ng/mL in Calibrator Diluent (1×) and assayed for cross-reactivity. Preparations of the following factors at 80 ng/mL in a mid-range recombinant mouse Cystatin C control were assayed for interference. No significant cross-reactivity or interference was observed.

Recombinant mouse:		**Recombinant human:**
Cathepsin A	Cathepsin L	Cathepsin O
Cathepsin B	Cathepsin Z	Cathepsin S
Cathepsin C	Cystatin A	Cystatin F
Cathepsin D	Cystatin B	Cystatin S
Cathepsin E	Cystatin E/M	Cystatin SA
Cathepsin H		Cystatin SN

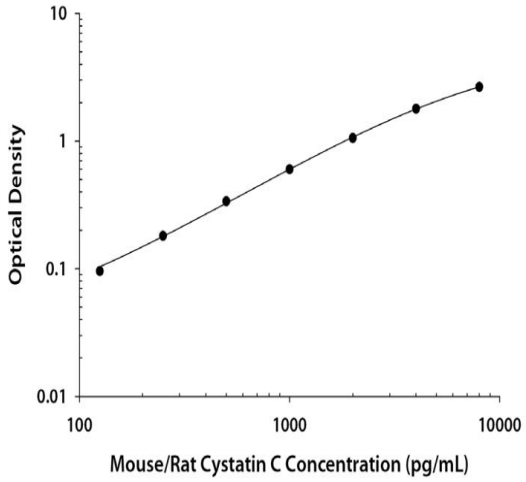
Recombinant human Cystatin C cross-reacts approximately 0.64% in this assay.

Recombinant human Cystatin D cross-reacts approximately 0.11% in this assay.

IV. EXPERIMENT

EXAMPLE STANDARD

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



(pg/mL)	O.D.	Average	Corrected
0	0.013 0.013	0.013	—
125	0.106 0.111	0.109	0.096
250	0.189 0.198	0.194	0.181
500	0.341 0.360	0.351	0.338
1000	0.603 0.625	0.614	0.601
2000	1.047 1.093	1.070	1.057
4000	1.802 1.804	1.803	1.790
8000	2.653 2.675	2.664	2.651

V. KIT COMPONENTS AND STORAGE

A. MATERIALS PROVIDED

Parts	Description	Size
Mouse/Rat Cystatin C Microplate	96 well polystyrene microplate (12 strips of 8 wells) coated with an antibody against mouse/rat Cystatin C.	1 plate
Mouse/Rat Cystatin C Conjugate	An antibody specific for mouse/rat Cystatin C conjugated to horseradish peroxidase.	1 vial
Mouse/Rat Cystatin C Standard	Recombinant mouse/rat Cystatin C in a buffered protein base; lyophilized. Refer to the vial label for reconstitution volume.	1 vial
Mouse/Rat Cystatin C Control	Recombinant mouse/rat Cystatin C in a buffered protein base; lyophilized. The assay value of the control should be within the range specified on the vial label.	1 vial
Assay Diluent RD1W	A buffered protein solution.	1 vial
Calibrator Diluent Concentrate (4×)/RD5-26	A 4× 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	Diluted hydrochloric acid.	1 vial
Plate Sealers	Adhesive strip.	3 strips

B. STORAGE

<table>
<tr><td>Unopened Kit</td><td colspan="2">Store at 2-8°C. Do not use past kit expiration date.</td></tr>
<tr><td rowspan="8">Opened/ Reconstituted Reagents</td><td>Wash Buffer (1×)</td><td rowspan="5">May be stored for up to 1 month at 2-8°C.*</td></tr>
<tr><td>Assay Diluent RD1W</td></tr>
<tr><td>Stop Solution</td></tr>
<tr><td>Conjugate</td></tr>
<tr><td>TMB Substrate</td></tr>
<tr><td>Control</td><td rowspan="2">May be stored for up to 1 month at 2-8 °C.*</td></tr>
<tr><td>Standard</td></tr>
<tr><td>Calibrator Diluent Concentrate (4×)/RD5-26</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.
- Test tubes for dilution of standards and samples.
- If using tissue lysate samples, the following is also required: Cell Lysis Buffer 2 (R&D Systems, Catalog # 895347).

D. PRECAUTION

- Cystatin C is detectable in saliva. Take precautionary measures to prevent contamination of kit reagents while running this assay.
- Some components in this kit contain a preservative which may cause an allergic skin reaction. Avoid breathing mist.
- The Stop Solution provided with this kit is an acid solution. Wear eye, hand, face, and clothing protection when using this material.

VI. PREPARATION

A. SAMPLE COLLECTION AND STORAGE

The sample collection and storage conditions listed below are intended as general guidelines. Sample stability has not been evaluated.

Cell Culture Supernates - Remove particulates by centrifugation and assay immediately or aliquot and store samples at ≤-20 °C. Avoid repeated freeze-thaw cycles. Samples may require dilution with Calibrator Diluent (1×).

Tissue Lysates - Cells must be lysed prior to assay as directed in the Sample Values section. Samples may require dilution with Calibrator Diluent (1×).

Serum - Allow blood samples to clot for 2 hours at room temperature before centrifuging for 20 minutes at 2000 × 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 EDTA or heparin as an anticoagulant. Centrifuge for 20 minutes at 2000 x g within 30 minutes of collection. Assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles. Samples may require dilution with Calibrator Diluent (1×).

Note: *Citrate plasma has not been validated for use in this assay. Grossly lipemic samples are not suitable for use in this assay.*

Urine - Collect urine using a metabolic cage. Remove any particulates by centrifugation and assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles. Centrifuge again before assaying to remove any additional precipitates that may appear after storage. Samples may require dilution with Calibrator Diluent (1×).

B. SAMPLE PREPARATION

Mouse serum and plasma samples, and rat urine samples recommend a 200-fold dilution into Calibrator Diluent (1×). A suggested 200-fold dilution is 10 µL of sample + 90 µL of Calibrator Diluent (1×). Complete the 200-fold dilution by adding 10 µL of the diluted sample + 190 µL of Calibrator Diluent (1×). Optimal dilutions should be determined by the end user.

Rat serum and plasma samples recommend a 400-fold dilution into Calibrator Diluent

(1×). A suggested 400-fold dilution is 10 µL of sample + 90 µL of Calibrator Diluent (1×). Complete the 400-fold dilution by adding 10 µL of the diluted sample + 390 µL of Calibrator Diluent (1×). Optimal dilutions should be determined by the end user.

Mouse urine samples recommend a 40-fold dilution into Calibrator Diluent (1×). A suggested 40-fold dilution is 10 µL of sample + 390 µL of Calibrator Diluent (1×). Optimal dilutions should be determined by the end user.

C. REAGENT PREPARATION

Bring all reagents to room temperature before use.

Note: *Cystatin C is detectable in saliva. It is recommended that a face mask and gloves be used to protect kit reagents from contamination.*

Mouse/Rat Cystatin C Control - Reconstitute the control with 1.0 mL of deionized or distilled water. Mix thoroughly. Assay the control undiluted.

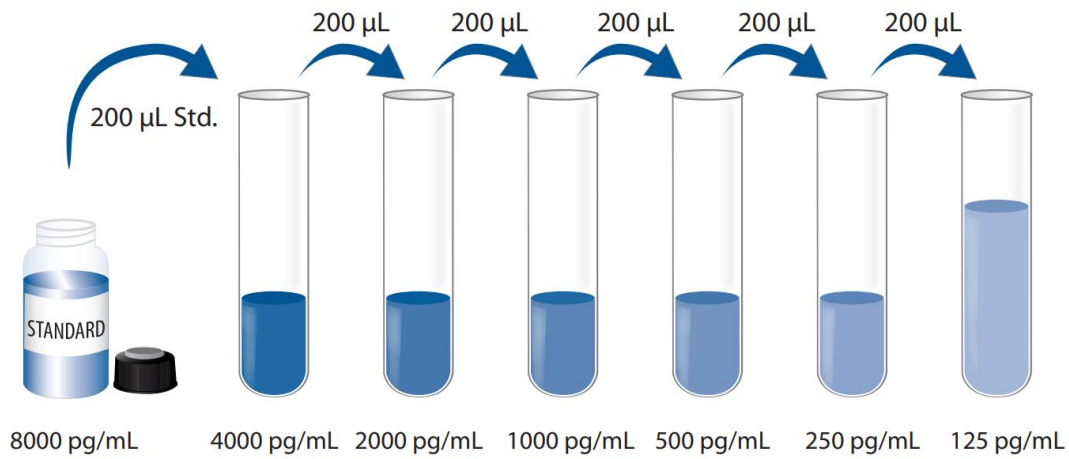
Wash Buffer (1×) - If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. Dilute 20 mL of Wash Buffer Concentrate (25×) into deionized or distilled water to prepare 500 mL of Wash Buffer (1×).

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

Mouse/Rat Cystatin C Standard- Refer to the vial label for the reconstitution volume* Reconstitute the Mouse/Rat Cystatin C Standard with Calibrator Diluent (1×). This reconstitution produces a stock solution of 8000 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 200 µL of Calibrator Diluent (1×) into each tube. Use the standard stock solution to produce a dilution series (below). Mix each tube thoroughly before the next transfer. The undiluted Mouse/Rat Cystatin C Standard (8000 pg/mL) 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 TMB Substrate. The color developed in the wells will turn from blue to yellow upon addition of the Stop Solution. Wells that are green in color indicate that the Stop Solution has not mixed thoroughly with the TMB Substrate.

VII.ASSAY PROCEDURE

Bring all reagents and samples to room temperature before use. It is recommended that all samples, control and standards be assayed in duplicate.
Note: *Cystatin C is detectable in saliva. It is recommended that a face mask and gloves be used to protect kit reagents from contamination.*

1. Prepare all reagents, working standards, control and samples as directed in the previous sections.
2. Remove excess microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, and reseal.
3. Add 50 µL of Assay Diluent RD1W to each well.
4. Add 50 µL of standard, control and prepared sample per well. Cover with the adhesive strip provided. **Incubate for 2 hours at room temperature.** A plate layout is provided for a record of standards and samples assayed.
5. Aspirate each well and wash, repeating the process four times for a total of five washes. Wash by filling each well with Wash Buffer (400 µL) using a squirt bottle, manifold dispenser, or autowasher. Complete removal of liquid at each step is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against clean paper towels.
6. Add 100 µL of Mouse/Rat Cystatin C 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. Gently tap the plate to ensure thorough mixing.
10. Determine the optical density of each well within 10 minutes, using a microplate reader set to 450 nm. If wavelength correction is available, set to 540 nm or 570 nm. If wavelength correction is not available, subtract readings at 540 nm or 570 nm from the readings at 450 nm. This subtraction will correct for optical imperfections in the plate. Readings made directly at 450 nm without correction may be higher and less accurate.
11. **CALCULATION OF RESULTS**

Average the duplicate readings for each standard, control and sample and subtract the average zero standard optical density (O.D.). Create a standard curve by reducing the data using computer software capable of generating a four-parameter logistic (4-PL) curve-fit. As an alternative, construct a standard curve by plotting the mean absorbance for each standard on the y-axis against the concentration on the x-axis and draw a best fit curve through the points on the graph. The data may be linearized by plotting the log of the mouse/rat 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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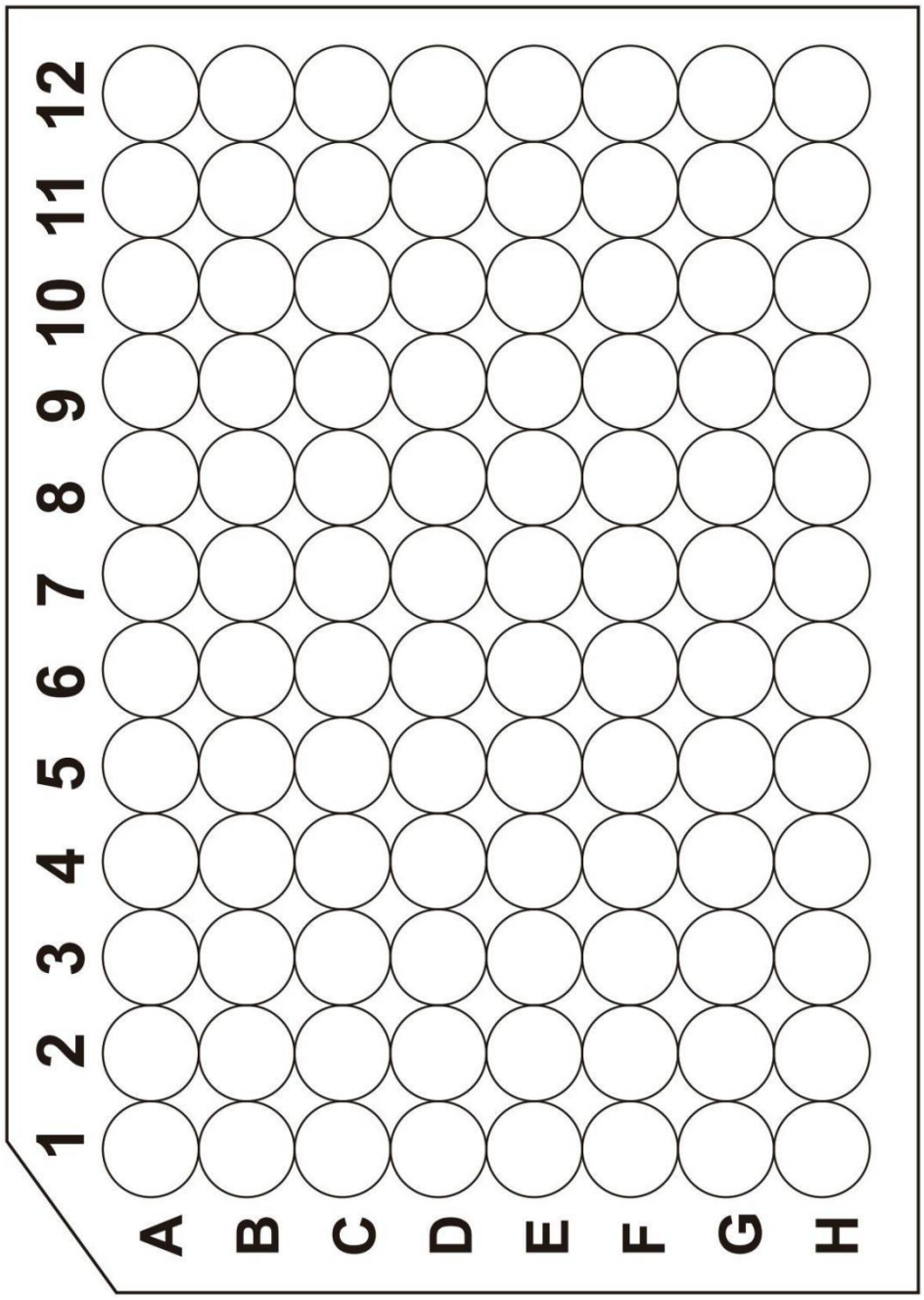
PLATE LAYOUT

Use this plate layout to record standards and samples assayed.



产品信息及操作手册

小鼠/大鼠 Cystatin C Valukine™ ELISA 试剂盒

目录号：VAL650

适用于定量检测天然和重组小鼠/大鼠胱抑素 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（基因名称 CST3）是一种分泌型细胞外半胱氨酸蛋白酶抑制剂，属于胱抑素超家族（1-4）。它是一种含有 120 个氨基酸（amino acids, aa）的蛋白质，非糖基化状态下约为 13 kDa；糖基化形式在大鼠中报道，但在小鼠中未报道（2-4）。小鼠和大鼠的胱抑素 C 与人类的胱抑素 C 有 72%的 aa 序列一致性，彼此之间具有 88%的 aa 序列一致性。胱抑素 C 易被内切蛋白酶裂解，产生 N 端截短形式（3, 4）。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，是胱抑素 C 的主要靶标（5, 6）。胱抑素 C 可在所有组织中产生，并存在于所有生物液体中。胱抑素 C 可由肾小球自由过滤。然后，胱抑素 C 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, 7-9）。因此，胱抑素 C 血清浓度与肾小球滤过率（glomerular filtration rate, GFR）密切相关。血清或血浆中胱抑素 C 的测量结果被认为是药物肾毒性的一个指标，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（1, 7, 9）。然而，在慢性低水平炎症期间，循环胱抑素 C 会增加，部分原因是 IL-6 介导的胱抑素 C 生成增加（1）。相反，抗炎细胞因子 IL-10、IFN-β和 IFN-γ可降低胱抑素 C 的表达及其循环水平（10-12）。

胱抑素 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，如果肾功能明显正常，循环胱抑素 C 却很高，这是冠状动脉和心血管疾病风险的一个指标（1, 9, 13, 14）。在人类主动脉瘤模型中，$ApoE^{-/-}$小鼠体内胱抑素 C 的缺失会促进炎症反应，并加速由酪蛋白酶介导的动脉壁弹力膜破裂（15, 16）。据报道，循环胱抑素 C 会影响肿瘤转移。异常低的胱抑素 C 含量导致组织蛋白 B 介导的细胞外基质降解并促进肿瘤转移，而高胱抑素 C 则会拮抗 TGF-β信号转导，减缓癌症的侵袭和生长（1, 17, 18）。胱抑素 C 是一种淀粉样蛋白。在人体生理条件下，L68Q 变体比野生型蛋白更容易形成二聚体和寡聚体，是遗传性胱抑素 C 淀粉样血管病的病因（5, 19, 20）。在阿尔茨海默病小鼠模型中，胱抑素 C 还能抑制淀粉样蛋白-β的沉积，保护神经细胞免受毒性影响（21-23）。

II. 概述

A. 检测原理

本实验采用双抗体夹心ELISA法。抗小鼠/大鼠Cystatin C抗体包被于微孔板上。样品，质控品和标准品中的小鼠/大鼠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小鼠/大鼠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样本，组织裂解物样本，小鼠/大鼠血清样本，小鼠/大鼠血浆样本和小鼠/大鼠尿液样本
- 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，在不同板间分别检测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)	434	894	3065	465	942	3257
标准差	14.9	29.2	85.9	43.5	51.2	213
CV%	3.4	3.3	2.8	9.4	5.4	6.5

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/大鼠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)	107	98-113

C. 灵敏度

41次检测结果表明，小鼠/大鼠Cystatin C的最低可测剂量（MDD）范围为2.47-12.9 pg/mL。平均MDD为3.93 pg/mL。

MDD是根据20个重复的零标准品孔的吸光度值的平均值加两倍标准差计算得到的相对应浓度。

D. 校正

该免疫测定法以R&D Systems生产的高纯度的NS0表达的重组小鼠/大鼠Cystatin C校正。

E. 线性

不同的样本中含有或掺入高浓度的小鼠/大鼠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。

小鼠样本		细胞培养上清(n=4)	组织裂解物(n=4)	血清(n=4)	EDTA血浆(n=4)	肝素血浆(n=4)	尿液(n=4)
1:2	平均值/期待值（%）	98	99	97	97	96	100
	范围（%）	87-101	97-100	96-98	93-100	94-101	99-101
1:4	平均值/期待值（%）	98	97	96	95	95	102
	范围（%）	86-106	94-100	90-100	90-100	89-103	99-107
1:8	平均值/期待值（%）	98	98	96	97	98	104
	范围（%）	88-110	93-103	87-101	92-103	94-108	103-104
1:16	平均值/期待值（%）	99	100	95	101	92	103
	范围（%）	90-111	90-109	89-104	91-110	89-94	98-108

大鼠样本		细胞培养上清(n=4)	血清(n=4)	EDTA血浆 (n=4)	肝素血浆(n=4)	尿液(n=4)
1:2	平均值/期待值（%）	92	93	97	91	94
	范围（%）	91-94	81-99	95-98	89-93	90-98
1:4	平均值/期待值（%）	88	91	98	86	90
	范围（%）	84-92	80-100	96-99	83-89	84-95
1:8	平均值/期待值（%）	86	92	98	85	89
	范围（%）	84-88	85-96	95-100	83-86	83-96
1:16	平均值/期待值（%）	80	93	97	85	88
	范围（%）	80-81	87-100	91-101	81-88	83-96

F. 样本预值

小鼠/大鼠血清/血浆/尿液样本 - 在此测定中对小鼠/大鼠样品进行小鼠/大鼠Cystatin C的可检测水平的评估。

小鼠样本	平均值 (ng/mL)	范围(ng/mL)	标准差(ng/mL)
血清样本 (n=20)	447	267-638	103
EDTA血浆样本 (n=20)	368	256-515	75.6
肝素血浆(n=20)	333	254-495	57.6
尿液(n=20)	119	7.45-241	60.4

大鼠样本	平均值 (ng/mL)	范围(ng/mL)	标准差(ng/mL)
血清 (n=20)	2111	1403-2678	360
EDTA 血浆 (n=20)	1542	963-1972	288
肝素血浆 (n=20)	1535	1002-2054	287
尿液 (n=20)	603	239-1383	316

细胞培养上清样本：

取出小鼠或大鼠的器官，置于冰上，用1×PBS冲洗，并保存在1×PBS中。将器官切成1-2 mm的小块，用组织匀浆器匀浆。然后培养在含10%胎牛血清、2 mM L-谷氨酰胺、100 U/mL青霉素和100 µg/mL硫酸链霉素的RPMI 1640培养基中。按下表所示培养细胞。取出等分的细胞培养上清，检测小鼠/大鼠Cystatin C。

小鼠组织	(ng/mL)
大脑 (1天)	70.1
心脏 (3天)	8.4
肾脏 (3天)	29.0
肝脏 (3天)	7.7
肺 (3天)	12.6
脾脏 (3天)	14.4

大鼠组织	(ng/mL)
大脑 (18小时)	225
心脏 (18小时)	10.2
肾脏 (18小时)	104
肺 (18小时)	31.1
脾脏(18小时)	8.6

将 3T3-L1 未分化小鼠胚胎成纤维细胞脂肪样细胞（2×10^6 cells/T75 flask）培养在含10% 胎牛血清、2 mM L-谷氨酰胺、100 U/mL青霉素和 100 μg/mL 硫酸链霉素的DMEM 培养基中，培养3天。去除培养基，加入50 mL新鲜培养基，再培养4天。取出等分的细胞培养上清液，检测小鼠/大鼠Cystatin C，检测值为166 ng/mL.

3T3-L1 分化的小鼠胚胎成纤维细胞脂肪样细胞（2×10^6 cells/T75 flask）培养在含10%胎牛血清、2 mM L-谷氨酰胺、100 U/mL青霉素和 100 μg/mL硫酸链霉素的DMEM培养基中 ，培养3天。去除培养基，加入10%胎牛血清、2 mM L-谷氨酰胺、100 U/mL青霉素、100 μg/mL 硫酸链霉素、1 μg/mL牛胰岛素、0.5 mM MIX和1 μM DEX培养基，再培养4天。取出等分的细胞培养上清液，检测小鼠/大鼠Cystatin C，检测值为507 ng/mL.

组织裂解物 - 用 1 × PBS 冲洗小鼠器官，然后用组织匀浆器在1 × PBS中匀浆。加入等体积的细胞裂解缓冲液2，在室温下温和搅拌30分钟，便于裂解组织。然后通过离心去除碎片。取出等分的组织裂解物，检测小鼠Cystatin C的水平。

小鼠组织	**(ng/mL)**
大脑	1254
心脏	210
肾脏	407
肝脏	26.7
肺	169
脾脏	127

G. 特异性

此ELISA法可检测天然及重组小鼠/大鼠Cystatin C 。

将以下因子用标准品稀释液(1×)配制成80 ng/mL的浓度来检测与小鼠/大鼠Cystatin C的交叉反应。将80 ng/mL的干扰因子掺入中间范围的重组小鼠/大鼠Cystatin C对照品中，来检测对小鼠/大鼠Cystatin C的干扰。没有观察到明显的交叉反应或干扰。

Recombinant mouse:		**Recombinant human**
Cathepsin A	Cathepsin L	Cathepsin O
Cathepsin B	Cathepsin Z	Cathepsin S
Cathepsin C	Cystatin A	Cystatin F
Cathepsin D	Cystatin B	Cystatin S
Cathepsin E	Cystatin E/M	Cystatin SA
Cathepsin H		Cystatin SN

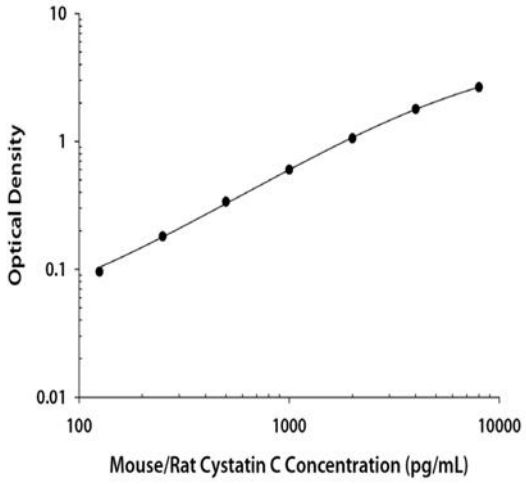
重组人Cystatin C在本试验中会有约0.64%的交叉反应。

重组人Cystatin D在本试验中会有约0.11%的交叉反应。

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)	O.D.	Average	Corrected
0	0.013 0.013	0.013	—
125	0.106 0.111	0.109	0.096
250	0.189 0.198	0.194	0.181
500	0.341 0.360	0.351	0.338
1000	0.603 0.625	0.614	0.601
2000	1.047 1.093	1.070	1.057
4000	1.802 1.804	1.803	1.790
8000	2.653 2.675	2.664	2.651

V. 试剂盒组成及储存

A. 试剂盒组成

组成	描述	规格
Mouse/Rat Cystatin C Microplate	包被抗小鼠/大鼠Cystatin C抗体的96孔聚苯乙烯板，8孔× 12条	1块板
Mouse/Rat Cystatin C Conjugate	酶标检测抗小鼠/大鼠Cystatin C抗体	1瓶
Mouse/Rat Cystatin C Standard	小鼠/大鼠Cystatin C标准品（冻干），参考瓶身标签进行重溶	1瓶
Mouse/Rat Cystatin C Control	小鼠/大鼠Cystatin C质控品（冻干），质控品的测定值应在标签上规定的范围内	1瓶
Assay Diluent RD1W	检测液	1瓶
Calibrator Diluent Concentrate (4×)/RD5-26	浓缩标准品稀释液(4×)用于稀释标准品和样本	1瓶
Wash Buffer Concentrate（25×）	浓缩洗涤缓冲液（25×）	1瓶
TMB Substrate	TMB ELISA底物溶液/TMB底物溶液	2瓶
Stop Solution	终止液	1瓶
Plate Sealers	封板膜	3张

B. 试剂盒储存

<table>
<tr><td>未开封试剂盒</td><td colspan="2">2-8℃储存；请在试剂盒有效期内使用</td></tr>
<tr><td rowspan="8">已打开，稀释或重溶的试剂</td><td>洗涤液（1×）</td><td rowspan="5">2-8℃储存，最多30天*</td></tr>
<tr><td>检测液RD1W</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>质控品</td><td rowspan="2">2-8℃储存，最多 30 天*</td></tr>
<tr><td>标准品</td></tr>
<tr><td>浓缩标准品稀释液(4×)/RD5-26</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
- 100 mL和500 mL量筒
- 用于稀释标准品和样品的管子。
- 如果使用组织裂解物样本，还需要以下材料： 细胞裂解缓冲液 2（R&D Systems，货号 # 895347）。

D. 注意事项

- 唾液中含有Cystatin C。在实验时，请采取预防措施，以防止试剂盒污染。
- 试剂盒中的一些组分含有防腐剂，可能引起皮肤过敏反应，避免吸入。
- 试剂盒中的终止液是酸性溶液，使用时请做好眼睛、手、面部及衣服的防护。

VI. 实验前准备

A. 样本收集及储存

以下列出的样品收集和储存条件仅作为一般指南。样本稳定性尚未评估。

细胞培养上清：颗粒物应通过离心去除；立即检测样本或分装，≤ -20 °C储存备用，避免反复冻融。样本可能需要用标准品稀释液(1×)稀释。

组织裂解物样本：检测前必须按照样本预值部分的说明裂解细胞。样品可能需要用标准品稀释液（1×）稀释。

血清样本：血液样本在室温下凝集2小时，然后在2000 × g下离心20分钟。吸取血清样本之后即刻用于检测，或者分装，≤ -20°C储存备用。避免反复冻融。样本可能需要用标准品稀释液(1×)稀释。

血浆样本：使用 EDTA或肝素作为抗凝剂收集血浆。然后2000 × g离心20分钟。需在30分钟内收集血浆样本之后即刻用于检测，或者分装，≤ -20°C储存备用。避免反复冻融。样本可能需要用稀释液(1×)稀释。

注意： *本试剂盒对枸橼酸钠血浆尚未被验证。*

本试剂盒不适合用于血脂过高样本。

尿液样本：使用代谢笼收集尿液。颗粒物应通过离心去除；立即检测样本或分装，≤ -20 °C储存备用，避免反复冻融。检测前再次离心，除去储存后可能出现的沉淀。样品可能需要用标准品稀释液（1×）稀释。

B. 样本准备工作

小鼠血清和血浆样本和大鼠尿液样本建议用标准品稀释液(1×) 200倍稀释后进行检测，即10 µL样品+90 µL标准品稀释液(1×)。再取10 µL稀释后样品+190 µL标准品稀释液（1×），即完成200倍稀释。最佳稀释度应由最终用户确定。

大鼠血清和血浆样本和建议用标准品稀释液(1×) 400倍稀释后进行检测，即10 µL样品+90 µL标准品稀释液(1×)。再取10 µL稀释后样品+ 390 µL 标准品稀释液（1×），即完成400倍稀释。最佳稀释度应由最终用户确定。

小鼠尿液样本建议用标准品稀释液(1×) 40倍稀释后进行检测，即10 µL样品+390 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。

注意：*唾液中含有Cystatin C。在检测时，建议使用手套及口罩以防试剂盒受到污染。*

小鼠/大鼠Cystatin C质控品：使用1.0 mL去离子水或蒸馏水重溶质控品。混合均匀，

测定时不稀释质控品。

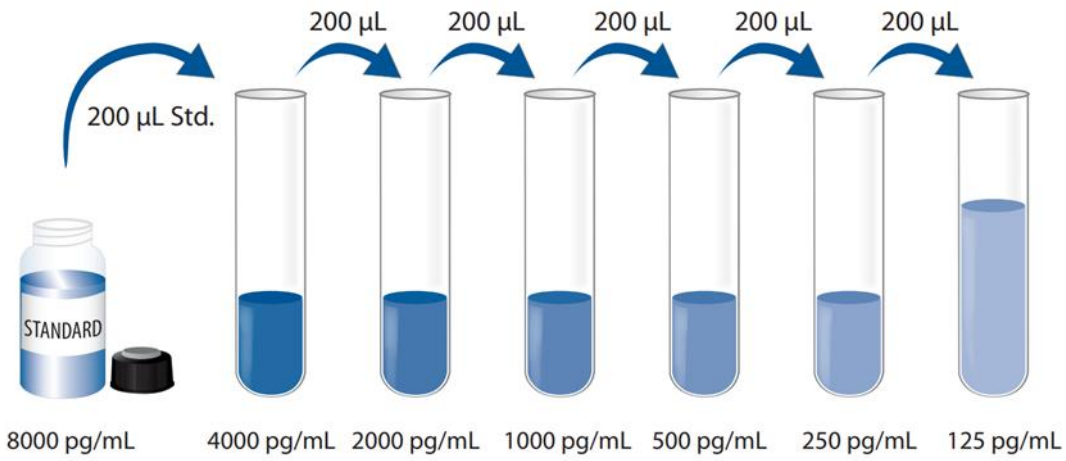
洗涤液（1×）：从冰箱中取出的浓缩洗涤液可能有结晶，属于正常现象；放置室温，轻摇混匀，待结晶完全溶解后再配制洗涤液。可将20 mL浓缩洗涤液（25×）用蒸馏水或去离子水稀释配制成500 mL工作浓度的洗涤液（1×）。

标准品稀释液（1×）：使用去离子水或蒸馏水配制标准品稀释液（1×）。

小鼠/大鼠Cystatin C标准品：重溶体积请参考瓶身标签*，用标准品稀释液(1×)重溶小鼠/大鼠Cystatin C标准品。得到浓度为8000 pg/mL标准品母液。轻轻震摇至少15分钟，其充分溶解。

*如有疑问，请咨询我们的技术支持。

向每一稀释管中加入200 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。未稀释的小鼠/大鼠Cystatin C标准品可用作标准曲线最高点（8000 pg/mL），标准品稀释液(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，配制不同浓度标准品、上样、加不同试剂都需要更换枪头。另外不同试剂请分别使用不同的移液槽；
- 建议15分钟内完成一块板的上样；
- 每次孵育时，正确使用封板膜可保证结果的准确性；
- TMB底物溶液在上板前应为无色，请避光保存；加入微孔板后，将由无色变成不同深度的蓝色；
- 终止液上板顺序应同TMB底物溶液上板顺序一致；加入终止液后，孔内颜色由蓝变黄； 若孔内有绿色，则表明孔内液体未混匀请充分混合；

VII. 操作步骤

使用前请将所有试剂和样本放置于室温。建议所有的实验样本，质控品和标准品做复孔检测。

注意：*唾液中含有Cystatin C。在检测时，建议使用手套及口罩以防试剂盒受到污染。*

1. 按照上一节的说明，准备好所有需要的试剂，标准品，质控品和样本；
2. 从已平衡至室温的密封袋中取出微孔板，未用的板条请放回铝箔袋内，重新封口；
3. 每孔加入50μL检测液RD1W。
4. 分别将不同浓度标准品，质控品和实验样本加入相应孔中，每孔50 μL。后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；
5. 将板内液体吸去，使用洗瓶、多通道洗板器或自动洗板机洗板。每孔加洗涤液400 μL，然后将板内洗涤液吸去。重复操作4次，共洗5次。每次洗板尽量吸去残留液体会有助于得到好的实验结果。最后一次洗板结束，请将板内所有液体吸干或将板倒置，在吸水纸拍干所有残留液体；
6. 在每个微孔内加入100 μL小鼠/大鼠Cystatin C酶标检测抗体。用封板膜封住反应孔，**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100 μL TMB底物溶液，**室温孵育30分钟。注意避光；**
9. 在每个微孔内加入100 μL终止液，请轻拍微孔板，使溶液混合均匀；
10. 加入终止液后10分钟内，使用酶标仪测量450 nm的吸光度值，设定540 nm或570 nm作为校正波长。如果波长校正不可用，以450 nm的读数减去540 nm或570 nm的读数。这种减法将校正酶标板上的光学缺陷。没有校正而直接在450 nm处进行的读数可能会更高且更不准确；
11. **计算结果：**将每个标准品，质控品和样品的复孔吸光值取平均值，然后减去零标准品平均OD值（O.D.），使用计算机软件作四参数逻辑（4-PL）曲线拟合创建标准曲线。另一替代方法是，通过绘制y轴上每个标准品的平均吸光值与x轴上的浓度来构建标准曲线，并通过图上的点绘制最佳拟合曲线。数据可以通过绘制小鼠/大鼠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