Human Cystatin C ELISA Kit

Catalog No.: BEK1039

Size: 96T

Range: 312 pg/ml - 20,000 pg/ml

Sensitivity < 10 pg/ml

Storage and Expiration: Store at 2-8℃ for 6 months, or at -20℃ for 12 months.

Application: For quantitative detection of Cystain C in human serum, plasma, body fluids, tissue lysates or cell culture supernatants.

Introduction

Cystatin C or cystatin 3, a protein encoded by the CST3 gene, is mainly used as a biomarker of kidney function. It is a single 120-residue polypeptide with a molecular mass of approximately 13.26 kD. Cystatin C plays a role in network reorganization in the epileptic dentate gyrus, especially in granule cell dispersion and guidance of migrating newborn granule cells. It is an alternative measure of kidney function that may have prognostic importance among elderly persons who do not meet standard criteria for chronic kidney disease. Among elderly persons without chronic kidney disease, Cystatin C is a prognostic biomarker of risk for death, cardiovascular disease, and chronic kidney disease, thereby signaling a state of 'preclinical' kidney dysfunction.

Principle of the Assay

This kit was based on sandwich enzyme-linked immune-sorbent assay technology. Anti-Cystatin C polyclonal antibody was pre-coated onto 96-well plates. And the biotin conjugated anti-Cystatin C polyclonal antibody was used as detection antibodies. The standards, test samples and biotin conjugated detection antibody were added to the wells subsequently, and wash with wash buffer. Avidin-Biotin-Peroxidase Complex was added and unbound conjugates were washed away with wash buffer. TMB substrates were used to visualize HRP enzymatic reaction. TMB was catalyzed by HRP to produce a blue color product that changed into yellow after adding acidic stop solution. The density of yellow is proportional to the Cystatin C amount of sample captured in plate. Read the O.D. absorbance at 450nm in a microplate reader, and then the concentration of Cystatin C can be calculated.

Kit components

1. One 96-well plate pre-coated with anti-Human Cystatin C antibody
2. Lyophilized Human Cystatin C standards: 2 tubes (20ng / tube)
3. Sample / Standard diluent buffer: 30ml
4. Biotin conjugated anti-Human Cystatin C antibody (Concentrated): 130μl. Dilution: 1:100
5. Antibody diluent buffer: 12ml
6. Avidin-Biotin-Peroxidase Complex (ABC) (Concentrated): 130μl. Dilution: 1:100
7. ABC diluent buffer: 12ml
8. TMB substrate: 10ml
9. Stop solution: 10ml
10. Wash buffer(25X): 30ml

Note: Reconstitute standards and test samples with Kit Component 3.

Material Required But Not Provided

1. 37℃ incubator
2. Microplate reader (wavelength: 450nm)
3. Precise pipette and disposable pipette tips
4. Automated plate washer
5. ELISA shaker
6. 1.5ml of Eppendorf tubes
7. Plate cover
8. Absorbent filter papers
9. Plastic or glass container with volume of above 1L

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Protocol

- **Preparation of sample and reagents**

1. Sample

Isolate the test samples soon after collecting, then, analyze immediately (within 2 hours). Or aliquot and store at -20℃ for long term. Avoid multiple freeze-thaw cycles.

✧ **Body fluids, tissue lysates and cell culture supernatants:** Centrifuge to remove precipitate, analyze immediately or aliquot and store at -20℃.

✧ **Serum:** Coagulate the serum at room temperature (about 2 hours). Centrifuge at approximately 2000-3000 × g for 10 min. Analyze the serum immediately or aliquot and store at -20℃.

✧ **Plasma:** Collect plasma with heparin or EDTA as the anticoagulant. Centrifuge for 15 min at 2000-3000 x g within 30 min of collection. Analyze immediately or aliquot and store frozen at -20°C. **Citrate can not be used as anticoagulant here.**

Note: 1. Coagulate blood samples completely, then, centrifuge, and avoid hemolysis and particle.

2. NaN_3 can not be used as test sample preservative, since it is the inhibitor for HRP.

>> Sample Dilution Guideline

End user should estimate the concentration of the target protein in the test sample first, and select a proper dilution factor to make the diluted target protein concentration falls the optimal detection range of the kit. Dilute the sample with the provided diluent buffer, and several trials may be necessary in practice. The test sample must be well mixed with the diluent buffer.

✧ **High target protein concentration (200-2000 ng/ml)**: Dilution: 1:100. i.e. Add 3μl of sample into 297 μl of Sample / Standard diluent buffer (Kit Component 3).

✧ **Medium target protein concentration (20-200 ng/ml)**: Dilution: 1:10. i.e. Add 25 μl of sample into 225 μl of Sample / Standard diluent buffer (Kit Component 3).

✧ **Low target protein concentration (312-20000 pg/ml)**: Dilution: 1:2. i.e. Add 100 μl of sample into 100 μl of Sample / Standard diluent buffer (Kit Component 3).

✧ **Very low target protein concentration (≤312 pg/ml)**: Unnecessary to dilute, or dilute at 1:2.

2. Wash buffer

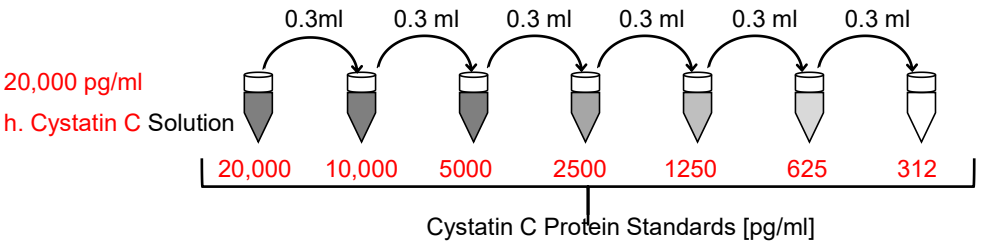
Dilute the concentrated Wash buffer 25-fold (1:25) with distilled water (i.e. add 30ml of concentrated wash buffer into 720ml of distilled water).

3. Standard

Reconstitution of the Lyophilized Human Cystatin C standard (Kit Component 2): standard solution should be prepared no more than 2 hours prior to the experiment. Two tubes of standard are included in each kit. Use one tube for each experiment. **(Note: Do not dilute the standard directly in the plate)**

a. 20,000 pg/ml of standard solution: Add **1 ml** of Sample / Standard diluent buffer (Kit Component 3) into one Standard (Kit Component 2) tube, keep the tube at room temperature for 10 min and mix thoroughly.

b. 10,000 pg/ml → 312 pg/ml of standard solutions: Label 6 Eppendorf tubes with 10,000 pg/ml, 5000 pg/ml, 2500 pg/ml,1250 pg/ml, 625 pg/ml, 312 pg/ml, respectively. Aliquot **0.3 ml** of the Sample / Standard diluent buffer (Kit Component 3) into each tube. Add **0.3 ml** of the above 20,000 pg/ml standard solution into 1st tube and mix thoroughly. Transfer **0.3 ml** from 1st tube to 2nd tube and mix thoroughly. Transfer **0.3 ml** from 2nd tube to 3rd tube and mix thoroughly, and so on.



Note: The standard solutions are best used within 2 hours. The 20,000 pg/ml standard solution should be used within 12 hours. Or store at -20℃ for up to 48 hours. Avoid repeated freeze-thaw cycles.

4. Preparation of Biotin conjugated anti-Human Cystatin C antibody (Kit Component 4) working solution: prepare no more than 2 hours before the experiment.

a. Calculate the total volume of the working solution: 0.1 ml / well × quantity of wells. (Allow 0.1-0.2 ml more than the total volume)

b. Dilute the Biotin conjugated anti-Human Cystatin C antibody (Kit Component 4) with Antibody diluent buffer (Kit Component 5) at 1:100 and mix thoroughly. i.e. Add 1 μl of Biotin conjugated anti-Human Cystatin C antibody into 99 μl of Antibody diluent buffer.

5. Preparation of Avidin-Biotin-Peroxidase Complex (ABC) (Kit Component 6) working solution: prepare no more than 1 hour before the experiment.

a. Calculate the total volume of the working solution: 0.1 ml / well × quantity of wells. (Allow 0.1-0.2 ml more than the total volume)

b. Dilute the Avidin-Biotin-Peroxidase Complex (ABC) (Kit Component 6) with ABC diluent buffer (Kit Component 7) at 1:100 and mix thoroughly. i.e. Add 1 μl of Avidin-Biotin-Peroxidase Complex (ABC) into 99 μl of ABC diluent buffer.

- **Assay procedure**

Before adding to wells, equilibrate the ABC working solution and TMB substrate (Kit Component 8) for at least 30 min at room temperature (37℃). It is recommend to plot a standard curve for each test.

1. Set standard, test sample and control (zero) wells on the pre-coated plate respectively, and then, record their positions. It is recommend to measure each standard and sample in duplicate.
2. Aliquot 0.1ml of 20,000 pg/ml, 10,000 pg/ml, 5000 pg/ml, 2500 pg/ml, 1250 pg/ml, 625 pg/ml, 312 pg/ml, standard solutions into the standard wells.
3. Add 0.1 ml of Sample / Standard diluent buffer (Kit Component 3) into the control (zero) well.
4. Add 0.1 ml of properly diluted sample (Human serum, plasma, body fluids, tissue lysates or cell culture supernatants) into test sample wells.
5. Seal the plate with a cover and incubate at 37℃ for 90 min.
6. Remove the cover and discard the plate content, clap the plate on the absorbent filter papers or other absorbent material. **Do NOT let the wells completely dry at any time. Do not wash plate!**
7. Add 0.1 ml of Biotin conjugated anti-Human Cystatin C antibody work solution into the above wells (standard, test sample & zero wells). Add the solution at the bottom of each well without touching the side wall.
8. Seal the plate with a cover and incubate at 37℃ for 60 min.

9. Remove the cover, and wash plate 3 times with Wash buffer (Kit Component 10) using one of the following methods:

   Manual Washing: Discard the solution in the plate without touching the side walls. Clap the plate on absorbent filter papers or other absorbent material. Fill each well completely with Wash buffer (Kit Component 10) and vortex mildly on ELISA shaker for 2 min, then aspirate contents from the plate, and clap the plate on absorbent filter papers or other absorbent material. Repeat this procedure two more times for a **total of THREE washes**.

   Automated Washing: Aspirate all wells, then wash plate **THREE times** with Wash buffer (Kit Component 10) (overfilling wells with the buffer). After the final wash, invert plate, and clap the plate on absorbent filter papers or other absorbent material. It is recommended that the washer be set for a soaking time of 1 min or shaking.
10. Add 0.1 ml of ABC working solution into each well, cover the plate and incubate at 37℃ for 30 min.
11. Remove the cover and wash plate 5 times with Wash buffer (Kit Component 10), and each time let the wash buffer stay in the wells for 1-2 min. (See Step 9 for plate wash method).
12. Add 0.1ml of TMB substrate (Kit Component 8) into each well, cover the plate and incubate at 37℃ in dark within 30 min. (**Note:** This incubation time is for reference use only, the optimal time should be determined by end user.) And the shades of blue can be seen in the first 3-4 wells (with most concentrated Human Cystatin C standard solutions), the other wells show no obvious color.
13. Add 0.1 ml of Stop solution (Kit Component 9) into each well and mix thoroughly. The color changes into yellow immediately.
14. Read the O.D. absorbance at 450 nm in a microplate reader within 30 min after adding the stop solution.

For calculation, (the relative $O.D._{450}$) = (the $O.D._{450}$ of each well) – (the $O.D._{450}$ of Zero well). The standard curve can be plotted as the relative $O.D._{450}$ of each standard solution (Y) vs. the respective concentration of the standard solution (X). The Human Cystatin C concentration of the samples can be interpolated from the standard curve.

Note: If the samples measured were diluted, multiply the dilution factor to the concentrations from interpolation to obtain the concentration before dilution.

Precautions

1. Before the experiment, centrifuge each kit component for several minutes to bring down all reagents to the bottom of tubes.
2. It is recommend to measure each standard and sample in duplicate.
3. Do NOT let the plate completely dry at any time! Since the dry condition can inactivate the biological material on the plate.
4. Do not reuse pipette tips and tubes to avoid cross contamination.
5. Do not use the expired components and the components from different batches.
6. To avoid the marginal effect of plate incubation for temperature differences (the marginal wells always get stronger reaction), it is recommend to equilibrate the ABC working solution and TMB substrate for at least 30 min at room temperature (37℃) before adding to wells.
7. The TMB substrate (Kit Component 8) is colorless and transparent before use, if not, please contact us for replacement.

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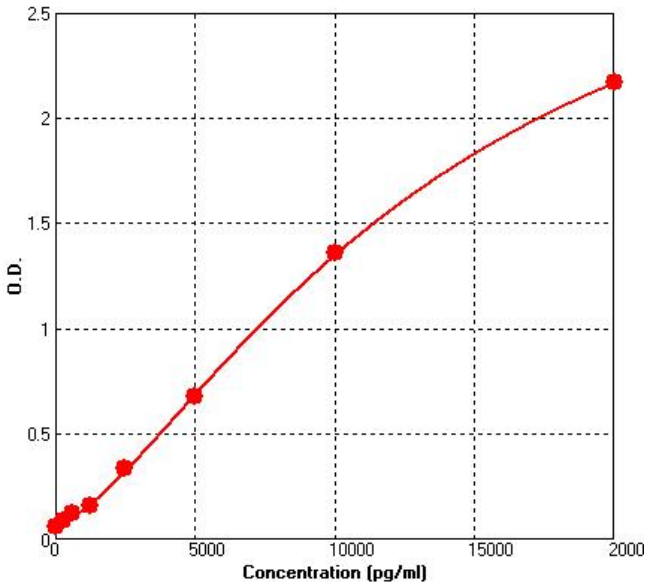
Typical Data & Standard Curve

Results of a typical standard run of a Human Cystatin C ELISA Kit are shown below. This standard curve was generated at our lab for demonstration purpose only. Each user should obtain their own standard curve as per experiment. (N/A=not applicable)

X	Pg/ml	0	312	625	1250	2500	5000	10,000	20,000
Y	OD450	0.058	0.092	0.124	0.157	0.338	0.681	1.357	2.167



Reference

1. Grubb, A., Lofberg, H. Human gamma-trace, a basic microprotein: amino acid sequence and presence in the adenohypophysis. Proc. Nat. Acad. Sci. 79: 3024-3027, 1982.

2. Pirttila, T. J., Manninen, A., Jutila, L., Nissinen, J., Kalviainen, R., Vapalahti, M., Immonen, A., Paljarvi, L., Karkola, K., Alafuzoff, I., Mervaala, E., Pitkanen, A. Cystatin C expression is associated with granule cell dispersion in epilepsy. Ann. Neurol. 58: 211-223, 2005.

3. Shlipak MG, Katz R, Sarnak MJ et al (August 2006). "Cystatin C and prognosis for cardiovascular and kidney outcomes in elderly persons without chronic kidney disease". Ann. Intern. Med. 145 (4): 237–46.

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