ImmunoDiagnostics Limited

FOR RESEARCH USE ONLY

Rapid Human Cystatin C ELISA Kit

(Catalog Number: 31241)

For the quantitative determination of human cystatin C concentrations in serum, plasma or urine samples

IMD (Hong Kong)
Address: Unit 513, 5/F, Biotech Centre 2, No. 11 Science Park West Avenue, Hong Kong Science Park, Sha Tin, Hong Kong
Website: www.immunodiagnostics.com.hk
Email: info@immunodiagnostics.com.hk
Tel: (+852) 3502 2780

IMD (Canada)
Address: 3330 Bayview Avenue, Block #6, Toronto, M2M 3R8, Ontario, Canada
Email: info@immunodiagnostics.ca
Tel: +1-437-886-5136

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INTRODUCTION

Human cystatin C (or cystatin 3), which is composed of 120 amino acid residues, belongs to the cystatins superfamily that inactivates lysosomal cysteine proteinases. As a strongly cationic and low-molecular weight (13.4 kDa) protein, it is almost freely filtered across the glomerular membrane, and is mainly used as a biomarker of kidney function. A growing body of evidence suggests that cystatin C is a more reliable biomarker of glomerular filtration rate than creatinine[1-3]. In addition to kidney disease, altered serum levels of cystatin C are associated with several types of cardiovascular disease, including myocardial infarction, stroke, heart failure, peripheral arterial disease and metabolic syndrome[4-7]. It also seems to play a role in brain disorders involving amyloid, such as Alzheimer's disease[8,9]. Furthermore, cystatin C has also been investigated as a prognostic marker in several forms of cancer[10-12].

PRINCIPLE OF THE ASSAY

This assay is a sandwich enzyme-linked immunosorbent assay (ELISA) designed for the quantitative detection of human cystatin C in samples in 1 hour. The microtiter plate is pre-coated with antibody specific to human cystatin C. Standards and samples are pipetted into the wells and any human cystatin C present is sandwiched by the immobilized antibody and a second horseradish peroxidase (HRP)-linked antibody specific to human cystatin C that is co-incubated with the samples. After wash step to remove any unbound substances, the HRP substrate solution is added and color develops in proportion to the amount of human cystatin C bound initially. The assay is stopped, and the optical density of the wells is determined using a micro-plate reader. Since the increases in absorbance are directly proportional to the amount of captured human cystatin C, the unknown sample concentration can be interpolated from a reference curve included in each assay.

INTENDED USE

This Rapid Human Cystatin C ELISA kit is designed for quantification of human cystatin C in serum, plasma and urine samples.

REAGENTS SUPPLIED

Each kit is sufficient for one 96-well plate and contains the following components:

1. Microtiter strips (96 wells), coated with antibody against human cystatin C
2. 10×Wash buffer, 30 mL
3. 5×Assay buffer, 30 mL
4. 100×Detection antibody solution, antibody against human cystatin C conjugated with horseradish peroxidase, 0.12 mL
5. Human cystatin C standard, 15 ng of native human cystatin C, lyophilized
6. Substrate solution, 12 mL, ready for use
7. Stop solution, 12 mL, ready for use
8. Plate cover

OTHER MATERIALS REQUIRED, BUT NOT PROVIDED

1. Pipettes and pipette tips
2. 96-well plate or manual strip washer
3. Buffer and reagent reservoirs
4. Paper towels or absorbent paper
5. Plate reader capable of reading absorbency at 450 nm
6. Distilled water or deionized water
7. Horizontal micro-plate shaker capable of 600 rpm

STORAGE

The kit should be stored at 2-8°C upon receipt, and all reagents should be equilibrated to room temperature before use. Remove any unused antibody-coated strips from the human cystatin C microtiter plate, return them to the foil pouch and re-seal. Once opened, the strips may be stored at 2-8°C for up to one month.

PREPARATION OF REAGENTS

Bring all reagents and materials to room temperature before assay.

A. 1×Assay buffer

Prepare 1×Assay buffer by mixing the 5×Assay buffer (30 mL) with 120 mL of distilled water or deionized water. If precipitates are observed in the 5×Assay buffer bottle, warm the bottle in a 37°C water bath until the precipitates disappear. The 1×Assay buffer may be stored at 2-8°C for up to one month.

B. 1×Wash buffer

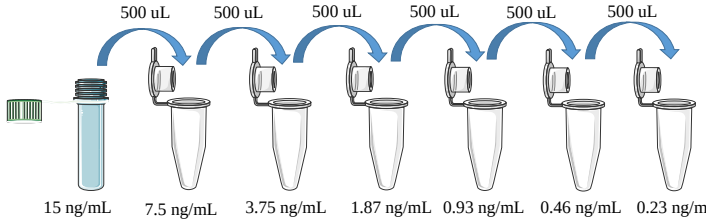
Prepare 1×Wash buffer by mixing the 10×Wash buffer (30 mL) with 270 mL of distilled water or deionized water. If precipitates are observed in the 10×Wash buffer bottle, warm the bottle in a 37°C water bath until the precipitates disappear. The 1×Wash buffer may be stored at 2-8°C for up to one month.

C. 1×Detection antibody solution

Spin down the 100×Detection antibody solution briefly and dilute the desired amount of the antibody 1:100 with 1×Assay buffer, 100 µL of the 1×Detection antibody solution is required per well. Prepare only as much 1×Detection antibody solution as needed. Return the 100×Detection antibody solution to 2-8°C immediately after the necessary volume is removed.

PREPARATION OF STANDARDS AND SAMPLES

Human Cystatin C Standards: Reconstitute the lyophilized standard with 1 mL of 1×Assay buffer to generate a standard stock solution of 15 ng/mL. Allow the standard to sit for 10 minutes with gentle agitation prior to making dilutions. Prepare serially diluted standards using 1×Assay buffer as shown below.



1×Assay buffer serves as the zero standard (0 ng/mL). The reconstituted standard stock should be aliquoted and frozen at -20ºC for one month. Avoid repeating freezing/thawing cycles. Please do not store the diluted standard solutions.

Sample Preparation:

Serum or plasma sample generally requires a **160-fold** dilution in this assay. A suggested dilution step is to add 5 µL of serum or plasma sample to 795 µL of 1×Assay buffer. Urine sample generally requires a **20-fold** dilution. A suggested dilution step is to add 50 µL of urine sample to 950 µL of 1×Assay buffer. It is recommended that the users establish their own dilution factors based on the concentration range of their samples.

ASSAY PROCEDURE

It is recommended that all standards and samples be assayed in duplicate.

1. Add 100 µL of standard or sample per well.
2. Add 100 µL of the 1×Detection antibody solution to each well, seal the plate with a plate cover. Incubate at room temperature for 30 minutes, shaking the plate at 600 rpm on a horizontal micro-plate shaker.
3. Discard the content and tap the plate on a clean paper towel to remove residual solution in each well. Add 300 µL of 1×Wash buffer to each well and incubate for 1 minute. Discard the 1×Wash buffer and tap the plate on a clean paper towel to remove residual wash buffer. Repeat the wash step for a total 4 washes.
4. Add 100 µL of Substrate solution to each well. Incubate at room temperature for 15 minutes. **Protect from light.**
5. Add 100 µL of Stop solution to each well, gently tap the plate frame for a few seconds to ensure thorough mixing.
6. Measure absorbance of each well at 450 nm immediately.

CALCULATION

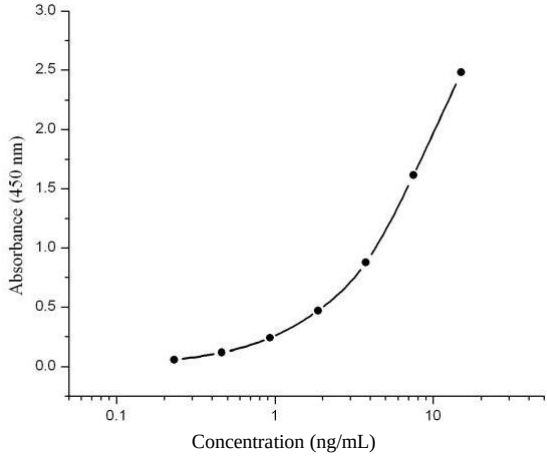
1. Subtract the absorbance of the blank from that of standards and samples.
2. Generate a standard curve by plotting the absorbance obtained (y-axis) against human cystatin C concentrations (x-axis). The best fit line can be generated with any curve-fitting software by regression analysis. Any curve of 4-parameter or log-log curve fitting can be used for calculation.
3. Determine human cystatin C concentration of samples from standard curve and multiply the value by the dilution factor.

TYPICAL STANDARD CURVE

The following standard curve is provided for demonstration only. A standard curve should be generated for each set of sample assay.

Cystatin C (ng/mL)	Absorbance (450 nm)	Blanked Absorbance
0	0.069	0
0.23	0.127	0.058
0.46	0.187	0.118
0.93	0.309	0.24
1.87	0.539	0.47
3.75	0.946	0.877
7.5	1.685	1.616
15	2.551	2.482

Human cystatin C standard curve (4-parameter)



ASSAY CHARACTERISTICS

A. Sensitivity

The lowest level of human cystatin C that can be detected by this assay is 0.23 ng/mL.

B. Specificity

The antibodies used in this assay are specific to human cystatin C and do not cross-react with mouse and rat cystatin C, and other cytokine or hormone molecules.

C. Precision

Intra-assay Precision (Precision within an assay)

Three samples of known concentration were tested 12 times on one plate.

Sample	Mean (ng/mL)	SD (ng/mL)	CV (%)
1	507.3	30.1	5.9
2	377.2	23.1	6.1
3	137.4	10.8	7.9

Inter-assay Precision (Precision between assays)

Three samples of known concentration were tested in 10 separate assays.

Sample	Mean (ng/mL)	SD (ng/mL)	CV (%)
1	499.3	39.0	7.8
2	377.2	23.1	6.1
3	137.4	10.8	7.9

D. Recovery

Serum samples were spiked with different amounts of human cystatin C and assayed.

Sample	Average % Recovery	Range %
Serum	99	94-107

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SUMMARY OF ASSAY PROCEDURE

Add 100 µL of standard or sample per well.
↓
Add 100 µL of 1×Detection antibody solution to each well.
↓
Incubate at room temperature for 30 minutes (600 rpm).
↓
Aspirate and wash each well 4 times.
↓
Add 100 µL of Substrate solution to each well.
↓
Incubate at room temperature for 15 minutes.
↓
Add 100 µL of Stop solution to each well.
↓
Measure absorbance of each well at 450 nm.
↓
Calculation