The Cystatin C Turbidimetric Immunoassay Kit

Catalogue number: 51240

For the quantitative determination of Cystatin C
in human serum, plasma and urine

This package insert must be read in its entirety before using this product
Use only the current version of product data sheet enclosed with the kit

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FOR RESEARCH USE ONLY
NOT FOR USE IN DIAGNOSTIC PROCEDURES

Version:6.1

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PACKING SPECIFICATION

Cat. No.	Size	Approximately tests
51240 -05	R1: 15ml, R2: 5ml	100
51240 -10	R1: 30ml, R2: 10ml	200
51240 -20	R1: 60ml, R2: 20ml	400
51240 -50	R1: 150ml, R2: 50ml	1000
51240 -100	R1: 300ml, R2: 100ml	2000

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 therefore 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. The normal range of Cystatin C in plasma sample is 0.5 – 10.3 mg/L. 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.

IMD developed its cystatin C PETIA kit with excellent reproducibility and accuracy equivalent to FDA-approved assays.

PRINCIPLE OF THE ASSAY

This assay is a turbidimetric immunoassay for the quantitative measurement of Cystatin C in human serum, plasma and urine. A standard or sample is added into a cuvette and mixed with the reaction buffer R1. After a short incubation, the test reagent R2, which is a suspension of microparticles coated with Cystatin C antibodies, is added into the cuvette and mixed. The presence of Cystatin C in the standard or sample causes the immune-particles to aggregate. The extent to which the microparticles aggregate is quantified by the amount of light scattering measured as absorbance by a chemistry analyzer. The concentration of Cystatin C in unknown samples can be interpolated from a reference curve using the standards provided.

REAGENTS SUPPLIED

R1 – Reaction buffer, a ready-to-use buffer solution containing salt, polyether compound and preservative

R2 – Test reagent, a ready-to-use suspension of polymer microparticles coated with rabbit anti- Cystatin C polyclonal antibodies in storage buffer

OTHER MATERIALS REQUIRED

1. Clinical chemistry analyzer
2. Cystatin C Calibrator (provided separately, Cat. #51240-S1)
3. Cystatin C Control (optional, provided separately, Cat. #51240-C1)
4. Deionized water
5. Analyzer-specific reagent containers for R1 and R2
6. Cystatin C calibrator set

STORAGE

The kit should be stored at 2-8°C upon receipt. Once opened, the reagents may be stored at 2-8°C for up to 4 weeks.

SPECIMEN COLLECTION AND HANDLING

This kit can be used to determine Cystatin C in human serum, plasma and urine samples. Blood specimens should be collected aseptically into appropriate tubes. Plasma should be prepared by standard techniques for laboratory testing. Urine should be centrifuged. The prepared specimens should be stored in closed vessels. If the assay cannot be performed within 24 hours or specimens are to be shipped, the specimens should be frozen at −20°C or below. For long-term storage of specimens, −70°C or below is recommended. To avoid freeze-thaw cycles, specimens should be aliquoted. Do not use hemolyzed, hyperlipemic, heat-treated or contaminated specimens. No dilution of the sample is required in this assay.

ASSAY PROCEDURE

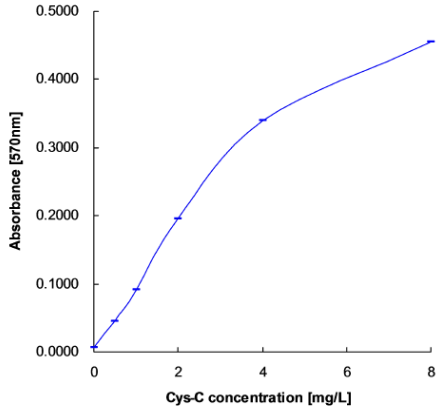
Assay procedures may vary depending on the automated chemistry analyzer to be used. A general example of assay procedures is stated as follow:

1. Dispense 150µl of R1 into a clean cuvette
2. Add 1.5µl of sample and incubate at 37˚C for 5 minutes
3. Further add 50µl of R2
4. Read change of absorbance at 570 nm for 8 minutes after the addition of R2
5. Calculate the concentration of Cystatin C in unknown sample by interpolation from a reference curve using the standards provided

TYPICAL STANDARD CURVE

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

Cystatin C (mg/L)	Absorbance (570 nm)
0	0.0072
0.5	0.0455
1	0.0920
2	0.1953
4	0.3395
8	0.4547



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 Cystatin C concentrations (x-axis). The best fit line can be generated with any curve-fitting software by regression analysis. 4-parameter curve fitting can be used for calculation.
3. Determine Cystatin C concentration of samples from standard curve.

PERFORMANCE CHARACTERISTICS

A. Sensitivity

The sensitivity is defined as the lower limit of detection and is estimated as the mean of the blank sample plus three times the SD obtained from the blank sample. The sensitivity of Cystatin C assay is 0.3 mg/L.

B. Precision

The precision of the Cystatin C assay is CV< 6%. Two serum Samples were assayed 20 times separately.

Sample	Mean Cystatin C (mg/L)	SD (mg/L)	CV
Panel 1	1.2	0.1	7.51%
Panel 2	4.2	0.2	3.54%

C. Linearity

The Cystatin C assay is linear between 0.5 mg/L and 8mg/L

D. Interference

No interference was detected with hemoglobin up to 5 g/L, conjugated bilirubin up to 300 mg/L, free bilirubin up to 300 mg/L, and up to 5g/L lipid emulsion.

E. Traceability:

Comparison of IMD Cystatin C assay to FDA-approved Cystatin C assay kit

	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	S11	S12
IMD results	2.40	1.37	4.73	1.21	1.24	5.84	1.33	5.75	4.86	3.29	1.64	2.18
Reference Value	2.37	1.33	4.95	1.26	1.33	5.86	1.41	6.16	5.25	3.26	1.63	2.17
Relative deviation	1.1 %	3.3 %	4.5 %	4.1 %	6.7 %	0.3 %	5.5 %	6.7 %	7.4 %	0.9 %	0.8 %	0.7 %
												R^2=0.9955

EXPECTED VALUES

Reference range: Plasma sample: 0.5 – 10.3 mg/L

WARNINGS AND PRECAUTIONS

- Do not pipette by mouth.
- Do not smoke, drink, or eat in areas where specimens or kit reagents are being handled.
- Wear protective clothing and disposable gloves while handling the kit reagents.
- Wash hands thoroughly after performing the test.
- Avoid contact with eyes; use safety glasses; in case of contact, flush with water immediately and contact a doctor.
- Avoid contact with skin; use gloves; in case of contact with skin, flush immediately and thoroughly with water.
- Dispose of all specimens and components of the kit as potentially infectious agents.
- Do not use the kit or any kit component past the indicated expiry date.
- Do not use any other reagents from different lots in this test, unless the reagent is designated to be used with other lots of the same kit.
- Do not use any reagent in other test kits, unless the reagent is designated to be used with other kits.
- Avoid microbial contamination of reagents.
- For manual pipetting of samples and controls, use individual pipette tips to eliminate carryover.

REFERENCES

1. Hojs R, Bevc S, et al. Serum cystatin C-based equation compared to serum creatinine-based equations for estimation of glomerular filtration rate in patients with chronic kidney disease. *Clin Nephrol.* 2008 Jul;70(1):10-7.