

Datasheet for ABIN577654

CST3 ELISA Kit[Go to Product page](#)**1** Image**3** Publications

Overview

Quantity: 96 tests

Target: CST3

Reactivity: Human

Method Type: Sandwich ELISA

Minimum Detection Limit: 0.171 ng/mL

Application: ELISA

Product Details

Purpose: The DetectX® Human Cystatin C kit is designed to quantitatively measure human Cystatin C present in biological samples and tissue culture media.

Brand: DetectX®

Sample Type: Serum, Plasma (EDTA), Plasma (heparin), Urine, Tissue Culture Medium

Analytical Method: Quantitative

Detection Method: Colorimetric

Specificity: Sample Types validated: Serum, EDTA and Heparin Plasma, Urine and Tissue Culture Media

Cross-Reactivity (Details): A serum sample was spiked with varying concentrations of bilirubin, diluted 1:50 in Assay Buffer and tested in the assay.

Bilirubin levels in normal serum are between 0.2 and 1.0 mg/ dL11.

At 10 times the highest concentration normally seen in human samples there was a 14 % decrease in measured Cystatin C concentration.

A serum sample was spiked with varying concentrations of hemoglobin in the form of RBCs,

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diluted 1:100 in Assay Buffer and tested in the assay.

No significant change to the measured Cystatin C level was observed.

A serum sample was spiked with varying concentrations of lipids, diluted 1:50 in Assay Buffer and tested in the assay.

No significant change to the measured Cystatin C level was observed with the addition of high, medium and low levels of lipids.

Dog, monkey, rat and mouse serum samples were serially diluted in the Assay Buffer and tested in the assay.

No Cystatin C was detected.

This kit should not be used for non-human samples.

Sensitivity: 0.058 ng/mL

Characteristics: The Cystatin C Immunoassay Kit is designed to measure Human Cystatin C levels in a variety of biological matrices. This kit uses a microtiter plate coated with a monoclonal to Cystatin C and a native human Cystatin C molecule standard. Samples and standards are added to the plate and after an hour incubation at RT the plate is washed and monoclonal antibody-peroxidase conjugate is added. After a 30-minute incubation at RT, the plate is washed and TMB substrate solution added to each well. The color reaction is read at 450nm. Cystatin C is a non-glycosylated protein of low molecular weight (13kDa) in the cystatin superfamily. Cystatin C is produced at a constant rate in all nucleated cells. Cystatin C belongs to the cysteine proteinase inhibitor group and is associated with several pathological conditions. Imbalance between Cystatin C and cysteine proteinases is associated with diseases such as inflammation, renal failure, cancer, Alzheimer's disease, multiple sclerosis and hereditary Cystatin C amyloid angiopathy. Cystatin C is removed from blood plasma by glomerular filtration in the kidneys. It is reabsorbed by the proximal tubular cells and degraded. There is a linear relationship between the reciprocal Cystatin C concentration in plasma and the glomerular filtration rate (GFR). Cystatin C is suggested to be a better marker for GFR than other markers as its serum concentration is not affected by other factors such as age, gender and body mass. There is association of Cystatin C levels with the incidence of myocardial infarction and coronary death, presenting a risk factor for secondary cardiovascular events.

Components: Clear Coated 96 Well Plate One Plate Clear plastic microplate with break-apart strips coated with mouse anti-human Cystatin C.

Cystatin C Standard 60 µL A stock solution of native human Cystatin C at 400 ng/mL.

DetectX® Cystatin C Conjugate 5 mL A monoclonal antibody to Cystatin C labeled with peroxidase.

Assay Buffer Concentrate 28 mL A 5X concentrate that should be diluted with deionized or

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distilled water.

Wash Buffer Concentrate 30 mL A 20X concentrate that should be diluted with deionized or distilled water.

TMB Substrate 5 mL

Stop Solution 5 mL A 1N hydrochloric acid solution. Caustic.

Plate Sealer 1 each

Material not included:

Distilled or deionized water.

A microplate washer.

Colorimetric 96 well microplate reader capable of reading optical density at 450 nm, preferably with correction between 570 and 590 nm.

Software for converting raw relative optical density readings from the plate reader and carrying out four parameter logistic curve (4PLC) fitting.

Contact your plate reader manufacturer for details.

Target Details

Target:

CST3

Alternative Name:

Cystatin C ([CST3 Products](#))

Background:

Cystatin C is a non-glycosylated protein of low molecular weight (13 kDa) in the cystatin superfamily. Cystatin C is produced at a constant rate in all nucleated cells, secreted from cells and thus found in detectable amounts in most body fluids^{1,2}. Cystatin C belongs to the cysteine proteinase inhibitor group and is associated with several pathological conditions. Imbalance between Cystatin C and cysteine proteinases is associated with diseases such as inflammation, renal failure, cancer, Alzheimer's disease, multiple sclerosis and hereditary Cystatin C amyloid angiopathy^{1,3,4}. Increased levels have been found in autoimmune diseases, with colorectal tumors and metastases, patients with inflammation and in patients on dialysis^{5,6,7}. Cystatin C is removed from blood plasma by glomerular filtration in the kidneys. It is reabsorbed by the proximal tubular cells and degraded. There is a linear relationship between the reciprocal Cystatin C concentration in plasma and the glomerular filtration rate (GFR). Cystatin C is suggested to be a better marker for GFR than the ubiquitous serum creatinine marker as its serum concentration is not affected by other factors such as age, gender and body mass and Cystatin C has higher sensitivity to detect a reduced GFR than creatinine determination^{2,5}. Low levels of Cystatin C are found with the breakdown of the elastic laminae and atherosclerosis and abdominal aortic aneurysm⁸. There is evident association of Cystatin C levels with the incidence of myocardial infarction, coronary death and angina pectoris,

Target Details

presenting a risk factor for secondary cardiovascular events⁹

Application Details

Application Notes:	<p>This assay has been validated for human serum, EDTA and heparin plasma, urine and tissue culture media (TCM) samples only.</p> <p>Samples containing visible particulate should be centrifuged prior to using.</p> <p>This assay has been shown to detect Cystatin C from human samples only.</p>
Comment:	<p>Sample values: Thirteen random human serum samples were tested in the assay.</p> <p>Values ranged from 532.0 to 905.6 ng/mL with an average of 675 ng/mL.</p> <p>The normal reference range for serum Cystatin C is 590-910 ng/mL¹⁰.</p> <p>An abnormal serum sample read at 1,384 ng/mL.</p>
Assay Time:	2 h
Plate:	Pre-coated
Protocol:	<p>A human Cystatin C standard is provided to generate a standard curve for the assay and all samples should be read off the standard curve.</p> <p>Standards or diluted samples are pipetted into a clear microtiter plate coated with a monoclonal antibody to capture the Cystatin C present.</p> <p>After a 60 minute incubation, the plate is washed and a peroxidase conjugated Cystatin C monoclonal antibody is added.</p> <p>The plate is again incubated for 30 minutes and washed.</p> <p>Sub- strate is then added to the plate, which reacts with the bound Cystatin C Antibody Conjugate.</p> <p>Af- ter a third incubation, the reaction is stopped and the intensity of the generated color is detected in a microtiter plate reader capable of measuring 450 nm wavelength.</p> <p>The concentration of the Cystatin C in the sample is calculated, after making suitable correction for dilution, using software available with most plate readers.</p>
Reagent Preparation:	<p>Allow the kit reagents to come to room temperature for 30 minutes.</p> <p>We recommend that all standards and samples be run in duplicate to allow the end user to accurately determine Cystatin C concentrations.</p> <p>Ensure that all samples have reached room temperature and have been diluted as appropriate prior to running them in the kit.</p> <p>Assay Buffer Dilute Assay Buffer Concentrate 1:5 by adding one part of the concentrate to four parts of deion- ized water.</p>

Once diluted this is stable at 4 °C for 3 months.

Wash Buffer Dilute Wash Buffer Concentrate 1:20 by adding one part of the concentrate to nineteen parts of deionized water.

Once diluted this is stable at room temperature for 3 months.

Standard Preparation Label seven glass test tubes as #1 through #7.

Briefly spin vial of standard in a microcentrifuge to ensure contents are at bottom of vial.

Pipet 585 µL of Assay Buffer into tube #1 and 250 µL into tubes #2 to #7.

Carefully add 15 µL of the Cystatin C stock solution to tube #1 and vortex completely.

Take 250 µL of the Cystatin C solution in tube #1 and add it to tube #2 and vortex completely.

Repeat the serial dilutions for tubes #3 through #7.

The concentration of Cystatin C in tubes #1 through #7 will be 10, 5, 2.5, 1.25, 0.625, 0.313 and 0.156 ng/mL.

Use all Standards within 2 hours of preparation.

Sample Preparation:

Serum and plasma samples must be diluted \geq 1:50 with the provided Assay Buffer prior to running in the kit. A dilution of \geq 1:225 is recommended to detect most samples within the standard curve range. Disease state samples, such as those from tubular kidney disease, may require further dilutions up to 1:500 or greater. It is up to the end user to determine the appropriate dilution for their samples. Urine samples must be diluted \geq 1:4 with the provided Assay Buffer prior to running in the kit. TCM samples should be diluted in TCM and read off a standard curve generated in the same TCM. Any samples with Cystatin C concentrations outside the standard curve range should be diluted further with Assay Buffer or TCM, as appropriate, to obtain readings within the standard curve. Use all samples within 2 hours of dilution.

Assay Procedure:

1. Use the plate layout sheet on the back page of the insert to aid in proper sample and standard identification. Determine the number of wells to be used and return unused wells to foil pouch with desiccant. Seal the ziploc plate bag and store at 4°C.
2. Pipet 50 µL of samples or standards into wells in the plate. Pipet 50 µL of Assay Buffer into the zero standard wells.
3. Incubate at room temperature for 60 minutes. Aspirate the plate and wash each well 4 times with 300 µL wash buffer. Tap the plate dry on clean absorbent towels.
4. Add 50 µL of the DetectX® Cystatin C Conjugate to each well, using a repeater pipet.
5. Incubate at room temperature for 30 minutes.
6. Aspirate the plate and wash each well 4 times with 300 µL wash buffer. Tap the plate dry on clean absorbent towels.
7. Add 50 µL of the TMB Substrate to each well, using a repeater pipet.

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8. Incubate the plate at room temperature for 30 minutes.
9. Add 50 μ L of the Stop Solution to each well, using a repeater pipet.
10. Read the optical density generated from each well in a plate reader capable of reading at 450 nm.
11. Use the plate reader's built-in 4PLC software capabilities to calculate Cystatin C concentration for each sample.

Calculation of Results:

Average the duplicate OD readings for each standard and sample.

Create a standard curve by reducing the data using computer software capable of generating a four-parameter logistic curve (4PLC) fit.

The sample concentrations should be multiplied by the dilution factor to obtain neat sample values.

Or use the online tool from <http://www.myassays.com/arbor-assays-cystatin-c-immunoassay-kit.assay> to calculate the data. *The MyAssays logo is a registered trademark of MyAssays Ltd.

Typical data

Sample	Mean OD	Human Cystatin C Conc. (ng/mL)
Standard 1	1.595	10
Standard 2	0.810	5
Standard 3	0.399	2.5
Standard 4	0.221	1.25
Standard 5	0.129	0.625
Standard 6	0.082	0.313
Standard 7	0.061	0.156
B0	0.042	0
Sample 1	0.636	3.76
Sample 2	0.094	0.40

Always run your own standard curve for calculation of results.

Do not use this data.

Restrictions:

For Research Use only

Handling

Precaution of Use:

As with all such products, this kit should only be used by qualified personnel who have had laboratory safety instruction.

The complete insert should be read and understood before attempting to use the product.

The antibody coated plate needs to be stored desiccated.

The silica gel pack included in the foil ziploc bag will keep the plate dry.

The silica gel pack will turn from blue to pink if the ziploc has not been closed properly.

The Cystatin C Standard is purified from a human source and as such, should be treated as potentially hazardous.

Proper safety procedures must be followed.

This kit utilizes a peroxidase-based readout system.

Buffers, including other manufacturers Wash Buffers, containing sodium azide will inhibit color production from the enzyme.

Make sure all buffers used for samples are azide free.

Ensure that any plate washing system is rinsed well with deionized water prior to using the supplied Wash Buffer as prepared on Page 8.

Handling

The Stop Solution is acid.

The solution should not come in contact with skin or eyes.

Take appropriate precautions when handling this reagent.

Storage: 4 °C,RT

Storage Comment: All components of this kit should be stored at 4 °C until the expiration date of the kit.

Publications

Product cited in: Villela-Torres, Higareda-Mendoza, Gómez-García, Alvarez-Paredes, García-López, Stenvikel, Gu, Rashid-Qureshi, Lindholm, Alvarez-Aguilar: "Copeptin Plasma Levels are Associated With Decline of Renal Function in Patients With Type 2 Diabetes Mellitus." in: **Archives of medical research**, (2018) ([PubMed](#)).

Barbati, Cappuccini, Aisa, Grasselli, Zamarra, Bini, Bellomo, Orlacchio, Di Renzo: "Increased Urinary Cystatin-C Levels Correlate with Reduced Renal Volumes in Neonates with Intrauterine Growth Restriction." in: **Neonatology**, Vol. 109, Issue 2, pp. 154-60, (2016) ([PubMed](#)).

Asnani, Reid: "Cystatin C: a useful marker of glomerulopathy in sickle cell disease?" in: **Blood cells, molecules & diseases**, Vol. 54, Issue 1, pp. 65-70, (2014) ([PubMed](#)).

Images

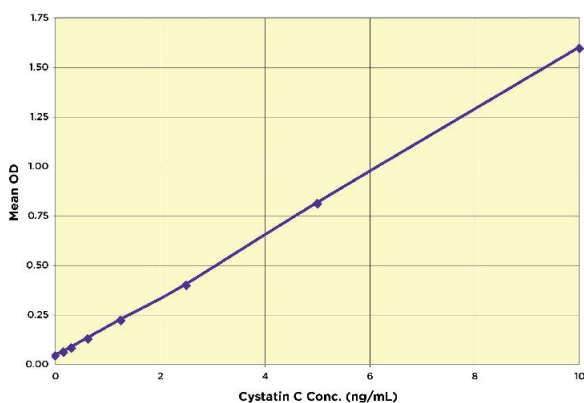


Image 1.