ARBOR ASSAYS™
Interactive Assay Solutions™

MOST SENSITIVE

DetectX®

Human Cystatin C
Enzyme Immunoassay Kit

1 Plate Kit Catalog Number K012-H1

New Standard Curve Range for Improved Sensitivity

Sample Types Validated:

Serum, EDTA and Heparin Plasma, Urine and Tissue Culture Media

Please read this insert completely prior to using the product.
For research use only. Not for use in diagnostic procedures.

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BACKGROUND

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, 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 aneurysm[8]. There is evident association of Cystatin C levels with the incidence of myocardial infarction, coronary death and angina pectoris, presenting a risk factor for secondary cardiovascular events[9].

1. Pergande M. and Jung K. "Sandwich Enzyme Immunoassay of Cystatin C in Serum with Commercially Available Antibodies." Clin Chem. 1993 39(9):1885-90.
2. Newman D, et al. "Serum cystatin C measured by automated immunoassay: A more sensitive marker of changes in GFR than serum creatinine." Kidney Int'l. 1995 Jan;47(1):312-8.
3. Levy E. "Cystatin C: a potential target for Alzheimer's treatment." Expert Rev. Neurotherapeutics 2008 May;8(5):687-9.
4. Nakashima I, et al. "Alteration of cystatin C in the cerebrospinal fluid of multiple sclerosis." Ann Neurol. 2007 Aug;62(2):197-200.
5. Uchida K. and Gotoh, A. "Measurement of cystatin C and creatinine in urine." Clin Chim Acta. 2002 Sep;323:121-8.
6. Pucci L, et al. "Cystatin C and Estimates of Renal Function: Searching for a Better Measure of Kidney Function in Diabetic Patients." Clin Chem. 2007 Mar;53(3):480-8.
7. Stabuc B, et al. "Improved Prediction of Decreased Creatinine Clearance by Serum Cystatin C: Use in Cancer Patients before and during Chemotherapy." Clin Chem. 2000 Feb;46(2):193-7.
8. Shlipak M, et al. "Cystatin C and the Risk of Death and Cardiovascular Events among Elderly Persons." NEJM 2005 May;352(20):2049-60.
9. Koenig W, et al. "Plasma Concentrations of Cystatin C in Patients with Coronary Heart Disease and Risk for Secondary Cardiovascular Events: More than Simply a Marker of Glomerular Filtration Rate." Clin Chem. 2005 Feb;51(2):321-7.

ASSAY PRINCIPLE

The DetectX® Human Cystatin C kit is designed to quantitatively measure human Cystatin C present in biological samples and tissue culture media. Please read the complete kit insert before performing this assay. 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. Standards or diluted samples are pipetted into a clear microtiter plate coated with a monoclonal antibody to capture the Cystatin C present. After a 60 minute incubation, the plate is washed and a peroxidase conjugated Cystatin C monoclonal antibody is added. The plate is again incubated for 30 minutes and washed. Substrate is then added to the plate, which reacts with the bound Cystatin C Antibody Conjugate. After 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. The concentration of the Cystatin C in the sample is calculated, after making suitable correction for dilution, using software available with most plate readers.

RELATED PRODUCTS

Kits	Catalog No.
Hemoglobin High Sensitivity Detection Kits	K013-HX1/HX5
Histone Demethylase Fluorescent Activity Kit	K010-F1
Retinol Binding Protein Multi-Format EIA Kits	K062-H1/H5
Serum Creatinine Detection Kits	KB02-H1/H2
Thiol Detection Kit	K005-F1
Urea Nitrogen (BUN) Detection Kits	K024-H1/H5
Urinary Creatinine Detection Kits	K002-H1/H5

SUPPLIED COMPONENTS

Clear Coated 96 Well Plate
Clear plastic microplate with break-apart strips coated with mouse anti-human Cystatin C.
One Plate — Catalog Number C033-1EA

Cystatin C Standard
A stock solution of native human Cystatin C at 400 ng/mL.
60 µL — Catalog Number C049-60UL

DetectX® Cystatin C Conjugate
A monoclonal antibody to Cystatin C labeled with peroxidase.
5 mL — Catalog Number C034-5ML

Assay Buffer Concentrate
A 5X concentrate that should be diluted with deionized or distilled water.
28 mL — Catalog Number X080-28ML

Wash Buffer Concentrate
A 20X concentrate that should be diluted with deionized or distilled water.
30 mL — Catalog Number X007-30ML

TMB Substrate
11 mL — Catalog Number X019-11ML

Stop Solution
A 1M hydrochloric acid solution. **CAUSTIC**.
5 mL — Catalog Number X020-5ML

Plate Sealer
1 each — Catalog Number X002-1EA

STORAGE INSTRUCTIONS

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

OTHER MATERIALS REQUIRED

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.

PRECAUTIONS

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.

The Stop Solution is acid. The solution should not come in contact with skin or eyes. Take appropriate precautions when handling this reagent.

SAMPLE TYPES

This assay has been validated for human serum, EDTA and heparin plasma, urine and tissue culture media (TCM) samples only. Samples containing visible particulate should be centrifuged prior to using.

This assay has been shown to detect Cystatin C from human samples only.

SAMPLE PREPARATION

Serum and plasma samples must be diluted ≥ 1:50 with the provided Assay Buffer prior to running in the kit. A dilution of ≥ 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 ≥ 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.

REAGENT PREPARATION

Allow the kit reagents to come to room temperature for 30 minutes. Ensure that all samples have reached room temperature and have been diluted as appropriate prior to running them in the kit.

Assay Buffer

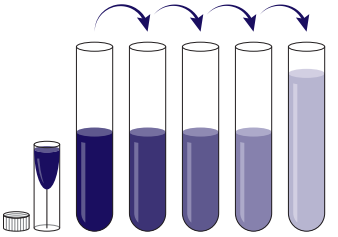
Dilute Assay Buffer Concentrate 1:5 by adding one part of the concentrate to four parts of deionized water. Once diluted this is stable at 4°C for 3 months.

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 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.

	Std 1	Std 2	Std 3	Std 4	Std 5	Std 6	Std 7
Assay Buffer Volume (µL)	**585**	250	250	250	250	250	250
Addition	Stock	Std 1	Std 2	Std 3	Std 4	Std 5	Std 6
Volume of Addition (µL)	**15**	250	250	250	250	250	250
Final Conc (ng/mL)	10	5	2.5	1.25	0.625	0.313	0.156

ASSAY PROTOCOL

We recommend that all standards and samples be run in duplicate to allow the end user to accurately determine Cystatin C concentrations.

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.
8. Incubate the plate at room temperature for 30 minutes.
9. Add 50 µL 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.

NOTE: If you are using only part of a strip well plate, at the end of the assay throw away the used wells and retain the plate frame for use with the remaining unused wells.

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.

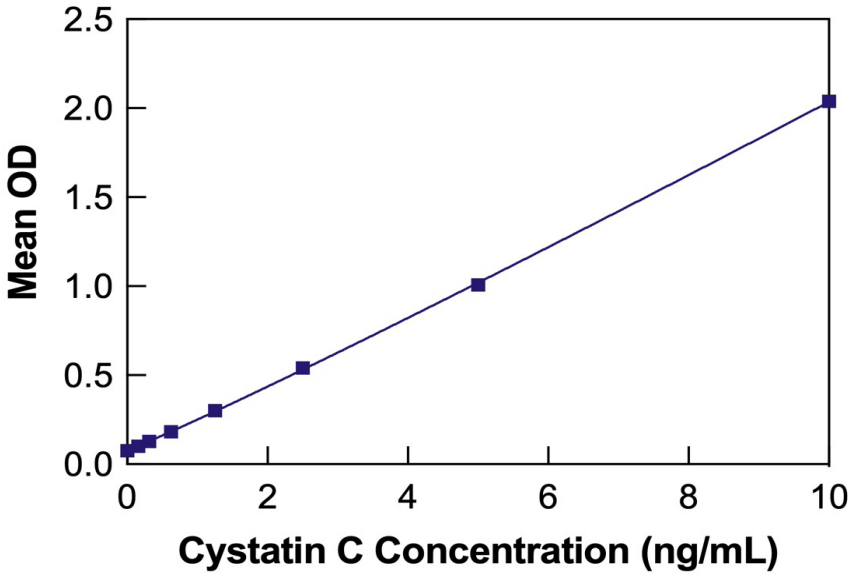
Or use the online tool from MyAssays to calculate the data:
www.myassays.com/arbor-assays-cystatin-c-immunoassay-kit.assay

TYPICAL DATA

Sample	Mean OD	Human Cystatin C Conc. (ng/mL)
Standard 1	2.038	10
Standard 2	1.008	5
Standard 3	0.540	2.5
Standard 4	0.302	1.25
Standard 5	0.182	0.625
Standard 6	0.128	0.313
Standard 7	0.102	0.156
B0	0.077	0
Sample 1	1.216	5.98
Sample 2	0.217	0.80

Always run your own standard curve for calculation of results. Do not use this data.

Typical Standard Curve



Always run your own standard curve for calculation of results. Do not use this data.

VALIDATION DATA

Sensitivity and Limit of Detection

Sensitivity was calculated by comparing the OD's for twenty wells run for each of the zero and standard #7. The detection limit was determined at two (2) standard deviations from the zero along the standard curve. **Sensitivity was determined as 0.058 ng/mL. This is equivalent to less than 3 pg/well.**

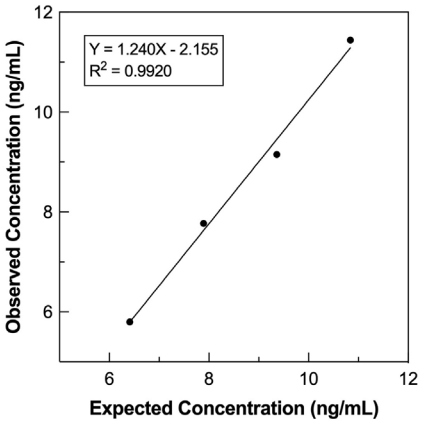
The Limit of Detection for the assay was determined in a similar manner by comparing the OD's for twenty replicates for each of the zero standard and a low concentration human serum sample. **Limit of Detection was determined as 0.171 ng/mL, equivalent to less than 9 pg/well.**

Linearity

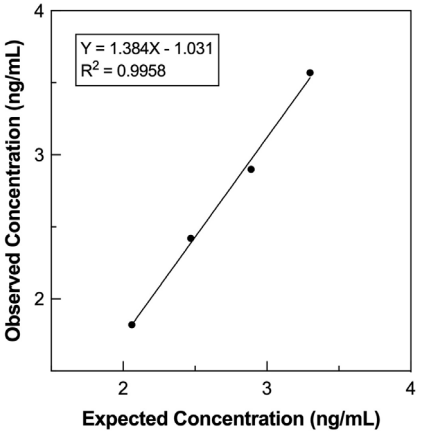
Serum linearity was determined by taking two human serum samples diluted 1:100, one with a low diluted Cystatin C level of 4.94 ng/mL and one with a higher diluted level of 12.31 ng/mL, mixing them in the ratios given below, and running in the assay. Urine linearity samples were diluted 1:16. One sample with a value of 1.64 ng/mL was mixed in the ratios below with a sample of 3.72 ng/mL. The measured concentrations were compared to the values previously determined.

High Sample	Low sample	Expected Conc. (ng/mL)		Observed Conc. (ng/mL)		% Recovery	
		Serum	Urine	Serum	Urine	Serum	Urine
80%	20%	10.84	3.30	11.44	3.57	105.6	108.1
60%	40%	9.36	2.89	9.15	2.90	97.7	100.4
40%	60%	7.89	2.47	7.77	2.42	98.5	97.9
20%	80%	6.41	2.06	5.80	1.82	90.4	88.5
				Mean Recovery		**98.1%**	**98.7%**

Serum Linearity



Urine Linearity



Intra Assay Precision

Four human samples were diluted with Assay Buffer and run in replicates of 20 in an assay. The mean and precision of the calculated Cystatin C concentrations were:

Sample	Cystatin C Conc. (ng/mL)	%CV
1	7.72	9.1
2	5.08	10.3
3	4.42	7.6
4	0.88	10.3

Inter Assay Precision

Four human samples were diluted with Assay Buffer and run in duplicates in ten assays run over multiple days by three operators. The mean and precision of the calculated Cystatin C concentrations were:

Sample	Cystatin C Conc. (ng/mL)	%CV
1	7.79	8.4
2	5.48	10.2
3	4.97	11.1
4	1.04	12.4

SAMPLE VALUES

Thirteen random human serum samples were tested in the assay. Values ranged from 532.0 to 905.6 ng/mL with an average of 675 ng/mL. The normal reference range for serum Cystatin C is 590-910 ng/mL[10]. An abnormal serum sample read at 1,384 ng/mL.

CROSS REACTIVITY AND INTERFERENTS

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/dL[11]. 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, 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.

10. "82994 Clinical: Cystatin C, Serum." Mayo Medical Laboratories: Reference Laboratory services for hospitals worldwide. 25 June 2009 www.mayomedicallaboratories.com/test-catalog/Clinical+and+Interpretive/82994
11. Tietz, N.W., in "Textbook of Clinical Chemistry", WB Sanders, 1986.

LIMITED WARRANTY

Arbor Assays warrants that at the time of shipment this product is free from defects in materials and workmanship. This warranty is in lieu of any other warranty expressed or implied, including but not limited to, any implied warranty of merchantability or fitness for a particular purpose.

We must be notified of any breach of this warranty within 48 hours of receipt of the product. No claim shall be honored if we are not notified within this time period, or if the product has been stored in any way other than outlined in this publication. The sole and exclusive remedy of the customer for any liability based upon this warranty is limited to the replacement of the product, or refund of the invoice price of the goods.

CONTACT INFORMATION

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OFFICIAL SUPPLIER TO ISWE

Arbor Assays and the International Society of Wildlife Endocrinology (ISWE) signed an exclusive agreement for Arbor Assays to supply ISWE members with EIA kits for wildlife conservation research.