QUANTITATIVE DETERMINATION OF HUMAN CYSTATIN C ELISA

IVD CE

Human Cystatin C ELISA

- High sensitivity (0.25 ng/ml)
- Excellent analytical characteristics
- Validated for human serum, urine, cerebrospinal fluid and plasma samples (EDTA, citrate, heparin)

RENAL DISEASE

BioVendor
Research
and Diagnostic Products

CYSTATIN C ELISA

Introduction

Cysteine proteinase inhibitors, cystatins superfamily, have been identified in animals, plants and protozoa. All cystatins inactivate lysosomal cysteine proteinases, e.g. cathepsin B, H, K, L and S as well as some structurally related plant proteinases, such as papain and actinidin. Human cystatin C is produced at a constant rate by all nucleated body cells and occurs in all body fluids abundantly. It is a non-glycosilated basic single-chain protein consisting of 120 amino acids with a molecular weight of 13.36 kDa and is characterized by two disulfide bonds in the carboxy-terminal region. The protein is encoded by the CS73 gene located on the short arm of chromosome 20.

Biological function of human cystatin C, and its role in various pathological states, has been the subject of numerous studies. Imbalance between cystatin C and cysteine proteinases is associated with diseases such as inflammation, renal failure, cancer, Alzheimer disease, multiple sclerosis and hereditary cystatin C amyloid angiopathy. Its increased level has been found in patients with autoimune diseases, with colorectal tumors and metastases, patients with inflammation and in patients on dialysis. Serum cystatin C concentration correlates negatively with glomerular filtration rate (GFR) as well as or better than creatinine, therefore was recently proposed as a new, very sensitive, marker of changes in GFR.

On the other hand, low levels of cystatin C come along the breakdown of the elastic laminae and, subsequently, the atherosclerosis and abdominal aortic aneurysm, as indicate latest publications. Results make evident association of cystatin C levels with the incidence of myocardial infarction, coronary death and angina pectoris. Furthermore, cystatin C correlates with triglycerides, LDL-cholesterol, BMI and age of individuals. Thus, low concentration of cystatin C presents a risk factor for secondary cardiovascular events.

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BioVendor Human Cystatin C ELISA (RD191009100)

Intended use

The RD191009100 Human Cystatin C ELISA is a sandwich enzyme immunoassay for the quantitative measurement of human cystatin C.

- **European Union: for *in vitro* diagnostic use. Rest of the world: for research use only!**
- The total assay time is less than 2 hours
- The kit measures total cystatin C in serum, plasma (EDTA, citrate, heparin), urine and cerebrospinal fluid
- Assay format is 96 wells
- Quality Controls are human serum or human urine native protein based. No animal sera are used
- Standard is purified native protein based
- Components of the kit are provided ready to use or concentrated

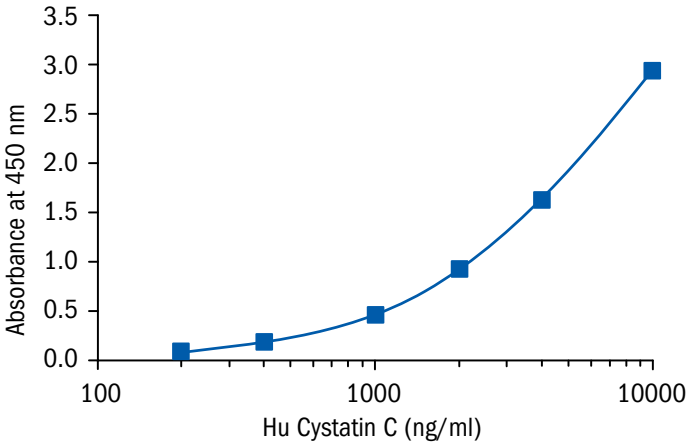
Test principle

In the BioVendor Human Cystatin C ELISA, standards, quality controls and samples are incubated in microtitrate plate wells pre-coated with polyclonal anti-human cystatin C antibody. After 30 minutes incubation and washing, polyclonal anti-human cystatin C antibody, conjugated with horseradish peroxidase (HRP) is added to the wells and incubated for 30 minutes with captured cystatin C. Following another washing step, the remaining HRP conjugate is allowed to react with the substrate solution (TMB). The reaction is stopped by addition of acidic solution and absorbance of the resulting yellow product is measured. The absorbance is proportional to the concentration of cystatin C. A standard curve is constructed by plotting absorbance values against concentrations of cystatin C standards, and concentrations of unknown samples are determined using this standard curve.

Clinical application

- Renal disease

HUMAN CYSTATIN C ELISA CAT. NO.: RD191009100	
Assay format	Sandwich ELISA, HRP-labelled antibody, 96 wells/kit
Samples	Serum, plasma (EDTA, heparin, citrate), urine, cerebrospinal fluid
Standards	200 to 10 000 ng/ml
Limit of detection	0.25 ng/ml



Precision

Intra-assay (Within-Run) (n=8)

Sample	Mean (ng/ml)	SD (ng/ml)	CV (%)
1	1 510	50	3.3
2	1 787	63	3.5

Inter-assay (Run-to-Run) (n=5)

Sample	Mean (ng/ml)	SD (ng/ml)	CV (%)
1	1 440	49	3.4
2	1 712	179	10.4

Spiking recovery

Serum samples were spiked with different amounts of human cystatin C, diluted with Dilution Buffer 400x and assayed.

Sample	Observed (ng/ml)	Expected (ng/ml)	Recovery O/E (%)
1	771	-	-
	1 146	1 171	98
	1 435	1 571	91
	2 702	2 771	98
2	978	-	-
	1 338	1 378	97
	1 566	1 778	88
	2 904	2 978	98

Linearity

Serum samples were serially diluted with Dilution Buffer after primary dilution 400x and assayed.

Sample	Dilution	Observed (ng/ml)	Expected (ng/ml)	Recovery O/E (%)
1	-	2 773	-	-
	2x	1 340	1 387	97
	4x	662	693	95
	8x	353	347	102
2	-	2 682	-	-
	2x	1 289	1 341	96
	4x	656	671	98
	8x	331	335	99

Effect of sample matrix

EDTA, citrate and heparin plasmas were compared to respective serum samples from the same 10 individuals.

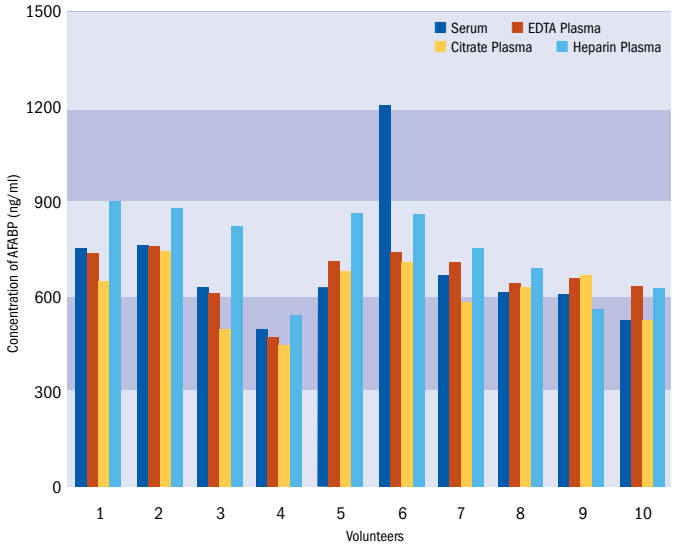


Figure: Cystatin C levels measured using Human Cystatin C ELISA from 10 individuals using serum, EDTA, citrate and heparin plasma, respectively.

QUANTITATIVE DETERMINATION OF HUMAN CYSTATIN C ELISA

Summary of protocol

- Reconstitute Master Standard and prepare set of Standards
- Dilute samples
- Add Standards, QCs and samples 100 µl
- Dilute serum/ plasma samples 400x, CSF samples 1 600x and/or urine samples 20x
- Dilute Standards and QCs 400x
- Incubate at RT for 30 min / 300 rpm
- Prepare Wash Solution
- Wash plate 3 times
- Prepare Conjugate Solution
- Add Conjugate Solution 100 µl
- Incubate at RT for 30 min / 300 rpm
- Wash plate 3 times
- Add 100 µl Substrate Solution
- Incubate at RT for 10 min
- Add 100 µl stop solution
- Read absorbance and calculate results

Related products

- RD491009100R Cystatin C Canine ELISA
- RD120009 Cystatin C Human PENTASET
- RD291009200R Cystatin C Mouse ELISA
- RD220009 Cystatin C Mouse PENTASET
- RD391009200R Cystatin C Rat ELISA
- RD320009 Cystatin C Rat PENTASET
- RD472009100 Cystatin C Canine E. coli
- RD172009100-H Cystatin C Human E. coli
- RD172009100 Cystatin C Human NATIVE, Human Urine
- RD272009100 Cystatin C Mouse E. coli
- RD184009100 Cystatin C (E.coli) Human, Sheep Polyclonal Antibody
- RD481009100 Cystatin C Canine, Rabbit Polyclonal Antibody
- RD484009100 Cystatin C Canine, Sheep Polyclonal Antibody
- RD181009220-01 Cystatin C Human, Rabbit Polyclonal Antibody
- RD181009220 Cystatin C Human, Rabbit Polyclonal Antibody
- RD184009220 Cystatin C Human, Sheep Polyclonal Antibody
- RD281009100 Cystatin C Mouse, Rabbit Polyclonal Antibody
- RD284009100 Cystatin C Mouse, Sheep Polyclonal Antibody
- RD384009100 Cystatin C Rat, Sheep Polyclonal Antibody

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Contact Information

BioVendor – Laboratorni medicina a.s.
Karasek 1767/1, 621 00 Brno, Czech Republic
Phone: +420 549 124 185, Fax: +420 549 211 460
E-mail: info@biovendor.com

BioVendor GmbH
Otto-Hahn-Straße 16, 34123 Kassel, Germany
Phone: +49 6221 4339 100, Fax: +49 6221 4339 111
E-mail: infoEU@biovendor.com

BioVendor GesmbH
Gaudenzdorfer Gürtel 43-45, 1120 Vienna, Austria
Phone: +43 1 890 9025, Fax: +43 1 890 5163
E-mail: infoAustria@biovendor.com

BioVendor, LLC
128 Bingham Rd., Suite 1300, Asheville, NC 28806, USA
Phone: +1-800-404-7807, Phone: +1-828-575-9250
Fax: +1-828-575-9251, E-mail: infoUSA@biovendor.com

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