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)
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 autoimmune 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.
**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

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**HUMAN CYSTATIN C ELISA**

**CAT. NO.: RD191009100**

<table>
<thead>
<tr>
<th>Component</th>
<th>Specification</th>
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<tbody>
<tr>
<td>Assay format</td>
<td>Sandwich ELISA, HRP-labelled antibody, 96 wells/kit</td>
</tr>
<tr>
<td>Samples</td>
<td>Serum, plasma (EDTA, heparin, citrate), urine, cerebrospinal fluid</td>
</tr>
<tr>
<td>Standards</td>
<td>200 to 10 000 ng/ml</td>
</tr>
<tr>
<td>Limit of detection</td>
<td>0.25 ng/ml</td>
</tr>
</tbody>
</table>

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**Absorbance at 450 nm**

Absorbance values are plotted against concentrations of cystatin C standards. The resulting curve is used to determine the concentration of cystatin C in unknown samples.
**Precision**

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

<table>
<thead>
<tr>
<th>Sample</th>
<th>Mean (ng/ml)</th>
<th>SD (ng/ml)</th>
<th>CV (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1510</td>
<td>50</td>
<td>3.3</td>
</tr>
<tr>
<td>2</td>
<td>1787</td>
<td>63</td>
<td>3.5</td>
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</table>

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

<table>
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<th>Sample</th>
<th>Mean (ng/ml)</th>
<th>SD (ng/ml)</th>
<th>CV (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1440</td>
<td>49</td>
<td>3.4</td>
</tr>
<tr>
<td>2</td>
<td>1712</td>
<td>179</td>
<td>10.4</td>
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</tbody>
</table>

**Spiking recovery**

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

<table>
<thead>
<tr>
<th>Sample</th>
<th>Observed (ng/ml)</th>
<th>Expected (ng/ml)</th>
<th>Recovery O/E (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>771</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>1146</td>
<td>1171</td>
<td>98</td>
</tr>
<tr>
<td></td>
<td>1435</td>
<td>1571</td>
<td>91</td>
</tr>
<tr>
<td></td>
<td>2702</td>
<td>2771</td>
<td>98</td>
</tr>
<tr>
<td>2</td>
<td>978</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>1338</td>
<td>1378</td>
<td>97</td>
</tr>
<tr>
<td></td>
<td>1566</td>
<td>1778</td>
<td>88</td>
</tr>
<tr>
<td></td>
<td>2904</td>
<td>2978</td>
<td>98</td>
</tr>
</tbody>
</table>

**Linearity**

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

<table>
<thead>
<tr>
<th>Sample</th>
<th>Dilution</th>
<th>Observed (ng/ml)</th>
<th>Expected (ng/ml)</th>
<th>Recovery O/E (%)</th>
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</thead>
<tbody>
<tr>
<td>1</td>
<td>-</td>
<td>2773</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>2x</td>
<td>1340</td>
<td>1387</td>
<td>97</td>
</tr>
<tr>
<td></td>
<td>4x</td>
<td>662</td>
<td>693</td>
<td>95</td>
</tr>
<tr>
<td></td>
<td>8x</td>
<td>353</td>
<td>347</td>
<td>102</td>
</tr>
<tr>
<td>2</td>
<td>-</td>
<td>2682</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>2x</td>
<td>1289</td>
<td>1341</td>
<td>96</td>
</tr>
<tr>
<td></td>
<td>4x</td>
<td>656</td>
<td>671</td>
<td>98</td>
</tr>
<tr>
<td></td>
<td>8x</td>
<td>331</td>
<td>335</td>
<td>99</td>
</tr>
</tbody>
</table>

**Effect of sample matrix**

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

![Effect of sample matrix graph](image)

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


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