CST3 (Human) ELISA Kit

Catalog Number KA0022

96 assays

Version: 12

Intended for research use only

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Introduction

Intended Use

The CST3 (Human) ELISA Kit is a sandwich enzyme immunoassay for the quantitative measurement of human cystatin C.

✓ Features

- 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

Background

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.

✓ Areas of investigation:

- Renal disease

Principle of the Assay

In the CST3 (Human) ELISA Kit, 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.

General Information

Materials Supplied

List of component

Component	Amount
Antibody Coated Microtiter Strips	96 wells
Conjugate Solution Conc. (50x)	0.26 mL
Conjugate Diluent (ready to use)	13 mL
Set of Standards (concentrated)	0.1 mL x 6
Quality Control HIGH (concentrated)	0.1 mL
Quality Control LOW (concentrated)	0.1 mL
Dilution Buffer Conc. (10 x)	10 mL
Wash Solution Conc. (10 x)	100 mL
Substrate Solution (ready to use)	13 mL
Stop Solution (ready to use)	13 mL

Storage Instruction

Store the complete kit at 2-8°C. Under these conditions, the kit is stable until the expiration date.

Materials Required but Not Supplied

- ✓ Deionized (distilled) water
- ✓ Test tubes for diluting samples
- ✓ Glassware (graduated cylinder and bottle) for Wash Solution (Dilution Buffer)
- ✓ Precision pipettes to deliver 10-1000 µL with disposable tips
- ✓ Multichannel pipette to deliver 100 µL with disposable tips
- ✓ Absorbent material (e.g. paper towels) for blotting the microtitrate plate after washing
- ✓ Vortex mixer
- ✓ Orbital microplate shaker capable of approximately 300 rpm
- ✓ Microplate washer (optional). [Manual washing is possible but not preferable.]
- ✓ Microplate reader with 450 ± 10 nm filter, preferably with reference wavelength 630 nm (alternatively another one from the interval 550 - 650 nm)
- ✓ Software package facilitating data generation and analysis (optional)

Precautions for Use

✓ Precautions

- For professional use only.
- Wear gloves and laboratory coats when handling immunodiagnostic materials.
- Do not drink, eat or smoke in the areas where immunodiagnostic materials are being handled.
- This kit contains components of human origin. These materials were found non-reactive for HBsAg, HCV antibody and for HIV 1/2 antigen and antibody. However, these materials should be handled as potentially infectious, as no test can guarantee the complete absence of infectious agents.
- Avoid contact with the acidic Stop Solution and Substrate Solution, which contains hydrogen peroxide and tetramethylbenzidine (TMB). Wear gloves and eye and clothing protection when handling these reagents. Stop and/or Substrate Solutions may cause skin/eyes irritation. In case of contact with the Stop Solution and the Substrate Solution wash skin/eyes thoroughly with water and seek medical attention, when necessary.
- The materials must not be pipetted by mouth.

✓ Technical hints

- Reagents with different lot numbers should not be mixed.
- Use thoroughly clean glassware.
- Use deionized (distilled) water, stored in clean containers.
- Avoid any contamination among samples and reagents. For this purpose, disposable tips should be used for each sample and reagent.
- Substrate Solution should remain colourless until added to the plate. Keep Substrate Solution protected from light.
- Stop Solution should remain colourless until added to the plate. The colour developed in the wells will turn from blue to yellow immediately after the addition of the Stop Solution. Wells that are green in colour indicate that the Stop Solution has not mixed thoroughly with the Substrate Solution.
- Dispose of consumable materials and unused contents in accordance with applicable national regulatory requirements

Assay Protocol

Reagent Preparation

- ✓ All reagents need to be brought to room temperature prior to use.
- ✓ Always prepare only the appropriate quantity of reagents for your test.
- ✓ Do not use components after the expiration date marked on their label.

- ✓ Assay reagents supplied ready to use:

- Antibody Coated Microtiter Strips

  Stability and storage:

  Return the unused strips to the provided aluminium zip-sealed bag with desicant and seal carefully. Remaining Microtiter Strips are stable 3 months stored at 2-8°C and protected from the moisture.
- Conjugate Diluent
- Substrate Solution
- Stop Solution

  Stability and storage:

  Opened reagents are stable 3 month when stored at 2-8°C

- ✓ Assay reagents supplied concentrated:

- Dilution Buffer Conc. (10x)

  Dilute Dilution Buffer Concentrate (10x) ten-fold in 90 mL distilled water to prepare a 1x working solution, e.g.10 mL of Dilution Buffer Concentrate (10x) + 90 mL of distilled water for use of all 96-wells.

  It is recommended to dilute only such a volume of Dilution Buffer Concentrate (10x) to be used up in the one run of the test.

  Stability and storage:

  The diluted Dilution Buffer is stable 1 week when stored at 2-8°C. Opened Dilution Buffer Concentrate (10x) is stable 3 months when stored at 2-8°C.
- Set of Standards

  Dilute each concentration of Standard 400x with the Dilution Buffer just prior to the assay in two steps as follows:

  Dilution A (10x):

  Add 10 µL of Standard into 90 µL of Dilution Buffer. Mix well (not to foam). Vortex is recommended.

  Dilution B (40x):

  Add 10 µL of Dilution A into 390 µL of Dilution Buffer to prepare final dilution (400x). Mix well (not to foam). Vortex is recommended.

  Stability and storage:

  Opened Standards are stable 3 months when stored at 2-8°C.

  Do not store the diluted Set of Standards.

- Quality Controls HIGH, LOW

  Refer to the Certificate of Analysis for current Quality Control concentration!!!

  Dilute each Quality Control (QC) 400x with the Dilution Buffer just prior to the assay in two steps as follows:

  Dilution A (10x):

  Add 10 µL of QC into 90 µL of Dilution Buffer. Mix well (not to foam). Vortex is recommended.

  Dilution B (40x):

  Add 10 µL of Dilution A into 390 µL of Dilution Buffer to prepare final dilution (400x). Mix well (not to foam). Vortex is recommended.

  Stability and storage:

  Opened Quality Controls are stable 3 months when stored at 2-8°C.

  Do not store the diluted Quality Controls.

  Note: Concentration of analyte in Quality Controls need not be anyhow associated with normal and/or abnormal concentrations in serum or another body fluid. Quality Controls serve just for control that the kit works in accordance with PDS and CoA and that ELISA test was carried out properly

  It is recommended to supplement two or three negative sample controls of customer's own (in addition to those provided with this kit). They can serve as evidence of the difference between positive and negative samples (see Figure 4 and Figure 5).

- Conjugate Solution Conc. (50x)

  Prepare the working Conjugate Solution by adding 1 part Conjugate Solution Concentrate (50x) with 49 parts Conjugate Diluent.

  Example: 0.25 mL of Conjugate Solution Concentrate (50x) + 12.25 mL of Conjugate Diluent for use of all 96-wells. Prepare only the volume needed for the test. Mix well (not to foam).

  Stability and storage:

  Opened Conjugate Solution Concentrate (50x) is stable 3 months when stored at 2-8°C.

  Do not store the diluted Conjugate Solution.

- Wash Solution Conc. (10x)

  Dilute Wash Solution Concentrate (10x) ten-fold in 900 mL of distilled water to prepare a 1x working solution, e.g. 100 mL of Wash Solution Concentrate (10x) + 900 mL of distilled water for use of all 96-wells.

  Stability and storage:

  The diluted Wash Solution is stable 1 month when stored at 2-8°C. Opened Wash Solution Concentrate (10x) is stable 3 months when stored at 2-8°C.

Sample Preparation

The kit measures cystatin C in serum, plasma (EDTA, citrate, heparin), urine and cerebrospinal fluid.
Samples should be assayed immediately after collection or should be stored at -20°C. Mix thoroughly thawed samples just prior to the assay and avoid repeated freeze/thaw cycles, which may cause erroneous results. Avoid using hemolyzed or lipemic samples.

Dilute samples (serum, plasma) 400x with the Dilution Buffer just prior to the assay in two steps as follows:
Dilution A (10x):
Add 10 µL of sample into 90 µL of Dilution Buffer. Mix well (not to foam). Vortex is recommended.
Dilution B (40x):
Add 10 µL of Dilution A into 390 µL of Dilution Buffer to prepare final dilution (400x). Mix well (not to foam). Vortex is recommended.

Dilute samples (CSF) 1600x with the Dilution Buffer just prior to the assay as follows:
Dilution A (40x):
Add 10 µL of sample into 390 µL of Dilution Buffer. Mix well (not to foam). Vortex is recommended.
Dilution B (40x):
Add 10 µL of Dilution A into 390 µL of Dilution Buffer to prepare final dilution (1600x). Mix well (not to foam). Vortex is recommended.

Stability and storage:
Samples should be stored at -20°, or preferably at -70°C for long-term storage. Avoid repeated freeze/ thaw cycles.
Do not store the diluted samples.

For dilution of urine samples see Urine cystatin C determination.

See Performance Characteristics for stability of serum, plasma and urine samples when stored at 2-8°C, effect of freezing/thawing and effect of sample matrix (serum/plasma) on the concentration of cystatin C.

Note: It is recommended to use a precision pipette and a careful technique to perform the dilution in order to get precise results.

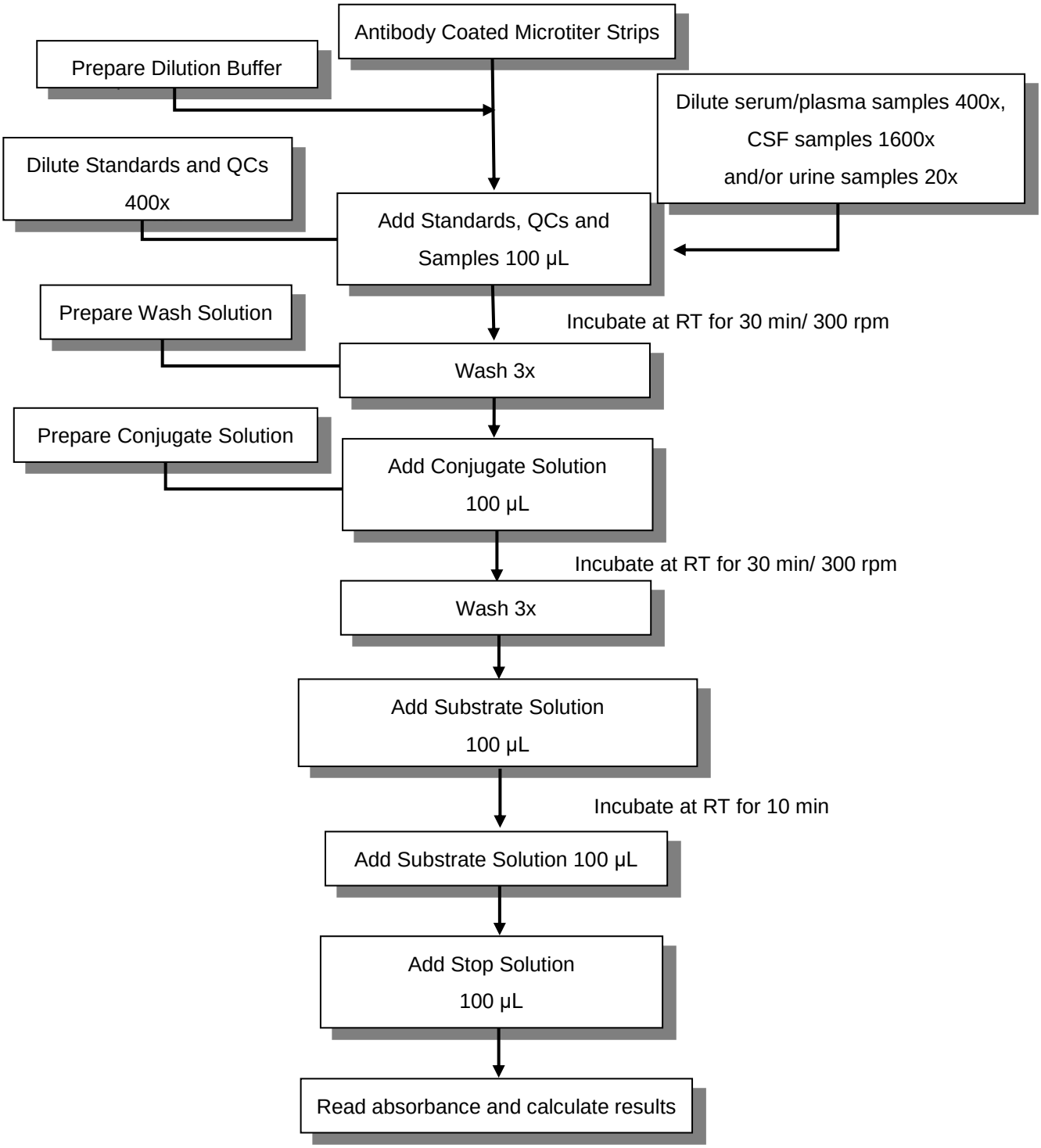
Assay Procedure

1. Pipet 100 µL of diluted Standards, Quality Controls, Dilution Buffer (=Blank) and samples, preferably in duplicates, into the appropriate wells. See Plate Layout for example of work sheet.
2. Incubate the plate at room temperature (ca. 25°C) for 30 minutes, shaking at ca. 300 rpm on an orbital microplate shaker.
3. Wash the wells 3-times with Wash Solution (0.35 mL per well). After final wash, invert and tap the plate strongly against paper towel.
4. Add 100 µL of Conjugate Solution into each well.
5. Incubate the plate at room temperature (ca. 25°C) for 30 minutes, shaking at ca. 300 rpm on an orbital microplate shaker.
6. Wash the wells 3-times with Wash Solution (0.35 mL per well). After final wash, invert and tap the plate strongly against paper towel.
7. Add 100 µL of Substrate Solution into each well. Avoid exposing the microtiter plate to direct sunlight. Covering the plate with e.g. aluminium foil is recommended.
8. Incubate the plate for 10 minutes at room temperature. The incubation time may be extended [up to 20 minutes] if the reaction temperature is below than 20°C. Do not shake with the plate during the incubation.
9. Stop the colour development by adding 100 µL of Stop Solution.
10. Determine the absorbance of each well using a microplate reader set to 450 nm, preferably with the reference wavelength set to 630 nm (acceptable range: 550 - 650 nm). Subtract readings at 630 nm (550 - 650 nm) from the readings at 450 nm.
    The absorbance should be read within 5 minutes following step 9.

Note: If some samples and standard/s have absorbances above the upper limit of your microplate reader, perform a second reading at 405 nm. A new standard curve, constructed using the values measured at 405 nm, is used to determine cystatin C concentration of off-scale standards and samples. The readings at 405 nm should not replace the readings for samples that were "in range" at 450 nm.

Note 2: Manual washing: Aspirate wells and pipet 0.35 mL Wash Solution into each well. Aspirate wells and repeat twice. After final wash, invert and tap the plate strongly against paper towel. Make certain that Wash Solution has been removed entirely.

✓ Assay Procedure Summary



Data Analysis

Calculation of Results

Most microplate readers perform automatic calculations of analyte concentration. The standard curve is constructed by plotting the mean absorbance (Y) of Standards against the known concentration (X) of Standards in logarithmic scale, using the four-parameter algorithm. Results are reported as concentration of cystain C ng/mL in samples.
Alternatively, the logit log function can be used to linearize the standard curve, i.e. logit of the mean absorbance (Y) is plotted against log of the known concentration (X) of Standards.
Use values of undiluted standard range: 10000, 4000, 2000, 1000, 400, 200 ng/mL.

Samples, Quality Controls and Standards are all diluted 400x prior to analysis, so there is no need to take this dilution factor into account.
Results are reported as total concentration of cystatin C (ng/mL) in serum/plasma samples. For the determination of concentration in samples diluted differently, use dilution factor for dividing/multiplying results read off the standard curve.

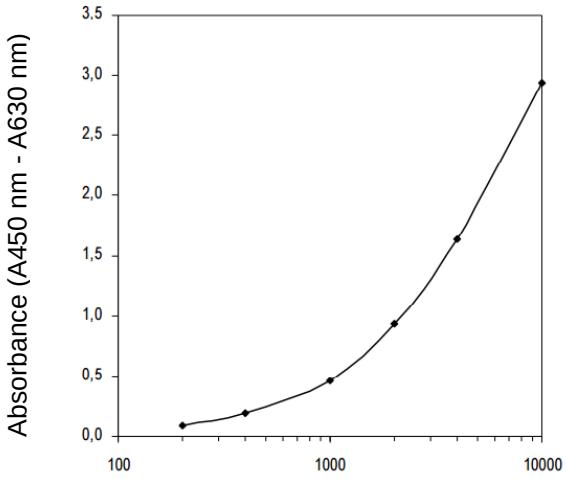


Figure 1: Typical Standard Curve for CST3 (Human) ELISA Kit.

Performance Characteristics

- ✓ Sensitivity

  Limit of Detection (LOD) (defined as concentration of analyte giving absorbance higher than mean absorbance of blank* plus three standard deviations of the absorbance of blank: $A_{blank} + 3xSD_{blank}$) is calculated from the real cystatin C values in wells and is 0.25 ng/mL.

  *Dilution Buffer is pipetted into blank wells.

✓ Limit of assay

Results exceeding cystatin C level of 10,000 ng/mL should be repeated with more diluted samples. Dilution factor needs to be taken into consideration in calculating the cystatin C concentration.

Example: Dilute samples 800x and dilution factor needs to be taken into consideration. The result (read off standard curve) is then multiplied by 2.

Conversely: If sample is diluted only 50x instead of 400x, due to lower concentration of analyte, the result (read off the standard curve) is divided by dilution factor 8, in this case.

Standard curve is plotted without changes, in both above mentioned cases, i.e. in undiluted concentrations: 10000, 4000, 2000, 1000, 400 and 200 ng/mL.

Note: cystatin C standard range 10,000-200 ng/mL, after 400x dilution, results in the actual concentration range 25-0.25 ng/mL, which represents concentration 2.5-0.025 ng/well. Thus, the assay system is capable of measuring these concentrations 25-0.25 ng/mL in 400x diluted samples, which can help to decide what dilution choose for samples other than sera.

✓ Specificity

The antibodies used in this ELISA are specific for human cystatin C.

Determination of cystatin C does not interfere with hemoglobin (1.0 mg/mL), bilirubin (170 µmol/L) and triglycerides (5.0 mmol/L).

Sera of several mammalian species were measured in the assay. See results below.

Mammalian serum sample	*Observed crossreactivity*
Bovine	no
Cat	no
Dog	no
Goat	no
Hamster	no
Horse	no
Monkey	yes
Mouse	no
Pig	no
Rabbit	no
Rat	no
Sheep	no

✓ Precision

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

Sample	Mean (ng/mL)	SD (ng/mL)	CV (%)
1	1510	50	3.3
2	1787	63	3.5

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

Sample	Mean(ng/mL)	SD (ng/mL)	CV (%)
1	1440	49	3.4
2	1712	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	-	-
	1146	1171	98
	1435	1571	91
	2702	2771	98
2	978	-	-
	1338	1378	97
	1566	1778	88
	2904	2978	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	-	2773	-	-
	2x	1340	1378	97
	4x	662	693	95
	8x	353	347	102
2	-	2682	-	-
	2x	1289	1341	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.

Results are shown below:

Volunteer No.	Serum (ng/mL)	Plasma (ng/mL)		
		EDTA	Citrate	Heparin
1	759	744	647	903
2	763	755	749	885
3	623	610	499	829
4	491	465	444	543
5	625	707	679	815
6	1206	737	712	862
7	676	706	574	753
8	619	646	624	690
9	605	669	668	570
10	527	631	528	619
Mean (ng/mL)	689	667	612	747
Mean Plasma/Serum (%)		97%	89%	108%

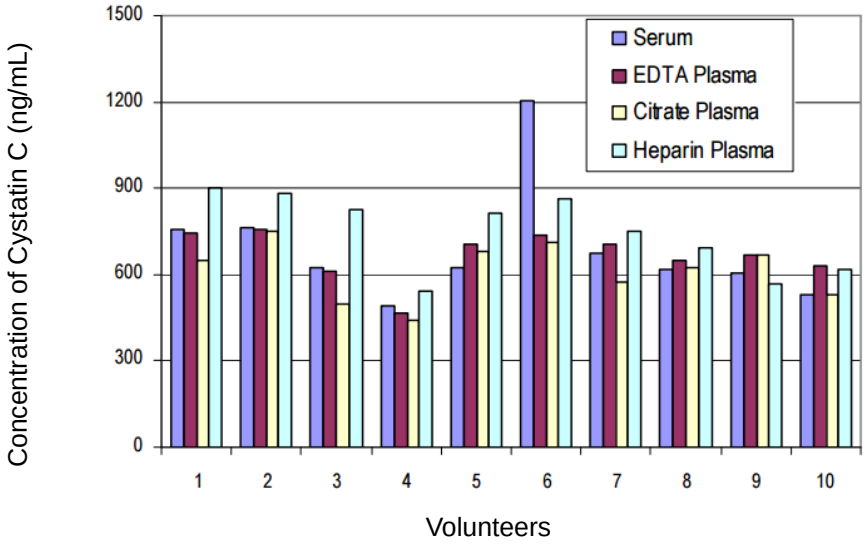


Figure 2: Cystatin C levels measured using CST3 (Human) ELISA Kit from 10 individuals using serum, EDTA, citrate and heparin plasma, respectively.

✓ Stability of samples stored at 2-8°C

Samples should be stored at -80°C. However, no decline in concentration of cystatin C was observed in serum and plasma samples after 7 days when stored at 2-8°C. To avoid microbial contamination, samples were treated with ε-aminocaproic acid and sodium azide, resulting in the final concentration of 0.03% and 0.1%, respectively.

Sample	Incubation Temp. Period	Serum (ng/mL)	Plasma (ng/mL)		
			EDTA	Citrate	Heparin
1	-80°C	1023	700	620	639
	2-8°C, 1 day	921	773	592	648
	2-8°C, 7 days	1171	762	615	647
2	-80°C	707	719	571	621
	2-8°C, 1 day	725	737	568	606
	2-8°C, 7 days	618	634	482	563
3	-80°C	625	660	483	603
	2-8°C, 1 day	639	637	499	620
	2-8°C, 7 days	636	651	552	603
4	-80°C	530	549	466	579
	2-8°C, 1 day	561	568	518	529
	2-8°C, 7 days	502	610	486	512

✓ Effect of Freezing/Thawing

No decline was observed in concentration of human cystatin C in serum and plasma samples after repeated (5x) freeze/thaw cycles. However it is recommended to avoid unnecessary repeated freezing/thawing of the samples.

Sample	Number of f/t cycles	Serum (ng/mL)	Plasma (ng/mL)		
			EDTA	Citrate	Heparin
1	1x	785	774	544	867
	3x	855	765	602	783
	5x	789	755	615	746
2	1x	599	721	613	719
	3x	549	715	531	734
	5x	632	676	632	740
3	1x	618	473	310	624
	3x	523	554	260	545
	5x	593	553	855	629
4	1x	387	518	394	454
	3x	370	411	354	442
	5x	461	465	349	497

✓ Definition of the Standard

The standard used in this kit is purified native protein based.

The standard used in the kit were calibrated against the European Reference Material ERM-DA 471/IFCC.

✓ Urine cystatin C determination

For the determination of cystatin C in urine use the serum/plasma protocol only with the following modifications:

- Sample collection and storage

  It is recommended to freeze down untreated urine although no significant decline was observed in concentration of human cystatin C in samples stored at 4°C for 14 days.

- Sample preparation

  Dilute urine samples 20x with Dilution Buffer just prior to use in the assay, e.g.: 20 µL of sample + 380 µL of Dilution Buffer.

  Stability and storage:

  Untreated urine samples are stable for 3 months when stored at -20°C/ -70°C.

  Do not store the diluted samples.

- Calculations of results

  Standard curve is plotted using values of undiluted Standards: 10000, 4000, 2000, 1000, 400 and 200 ng/mL. As urine samples are diluted only 20x whereas Standards are diluted 400x, the result (read off the Standard curve) has to be divided by dilution factor 20 in order to obtain the real concentration in the original (undiluted) sample.

- Effect of freezing/thawing on the concentration of cystatin C in urine

  Cystatin C levels were determined in the morning urine from fifteen individuals who were examined because of a suspicion of renal dysfunction. All of them had urine protein < 0.3 g/day and a normal count of leukocytes in urine.

  Assay results are shown below:

Sample No.	Cystatin C (ng/mL)	
	1x F/T	5x F/T
1	31	33
2	62	66
3	30	22
4	11	13
5	24	24
6	22	24
7	48	42
8	32	30
9	27	32
10	101	95

11	39	41
12	51	63
13	10	8
14	84	86
15	47	43

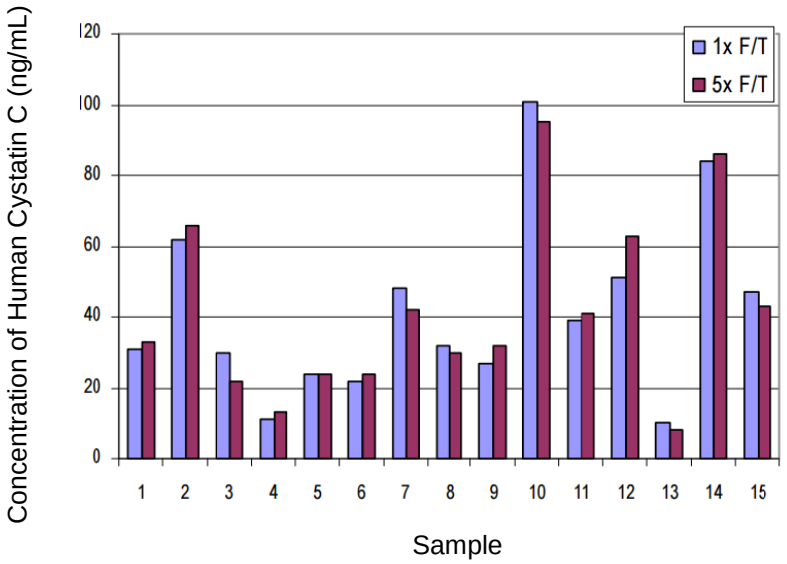


Figure 3: Cystatin C concentration was determined in urine after repeated freeze-thaw cycles. Samples were taken from fifteen individuals who were suspected to have renal dysfunction.

✓ Preliminary population data

The following results were obtained when serum samples from 155 unselected individuals (89 men + 66 women) 21 - 65 years old were assayed with CST3 (Human) ELISA Kit.

Sex	Age (years)	n	Cystatin C (ng/mL)				
			Mean	Median	SD	Min	Max
Men	20-29	17	1191.2	952.9	548.3	477.4	2225.0
	30-39	25	1204.0	1211.4	434.9	275.2	2038.5
	40-49	31	1093.6	1018.4	397.6	414.0	2353.4
	50-65	16	1208.0	960.2	639.0	529.3	3175.3
Women	20-29	12	930.2	1010.3	279.9	442.1	1263.7
	30-39	26	1082.2	1040.0	334.2	555.0	1687.5
	40-49	20	878.4	808.9	321.7	501.0	1794.4
	50-61	8	984.9	919.7	320.3	693.1	1687.5

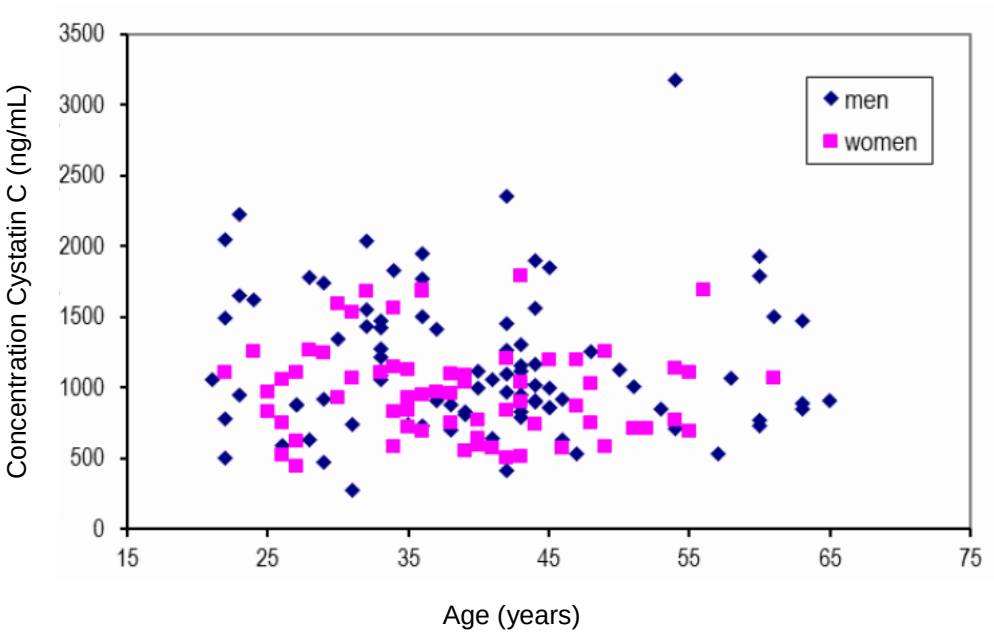


Figure 4: Human Cystatin C concentration plotted against individuals age and sex.

✓ Serum cystatin C determination

Sera from eight individuals on long-term dialysis were measured and their cystatin C levels compared to control sera from ten normal, apparently healthy individuals:

Sample No.	Cystatin C (ng/mL)	CV (%)
1	8335	6
2	8014	8
3	6822	1
4	9464	8
5	7844	8
6	3366	4
7	5955	1
8	3583	14

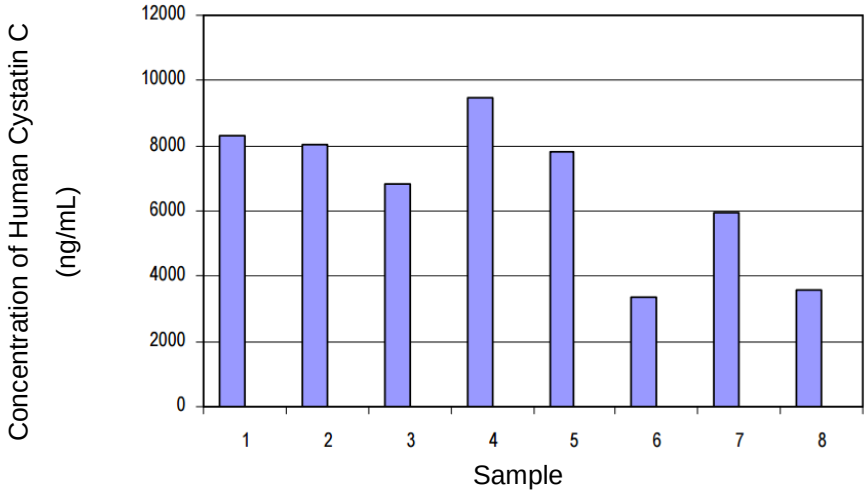


Figure 5: Cystatin C concentration was determined in serum samples from eight individuals on long-term dialysis.

Sample No.	Cystatin C (ng/mL)	CV (%)
Pooled serum	1032	11
1	885	9
2	979	4
3	703	8
4	1178	6
5	943	8
6	751	9
7	850	5
8	1532	6
9	1328	2

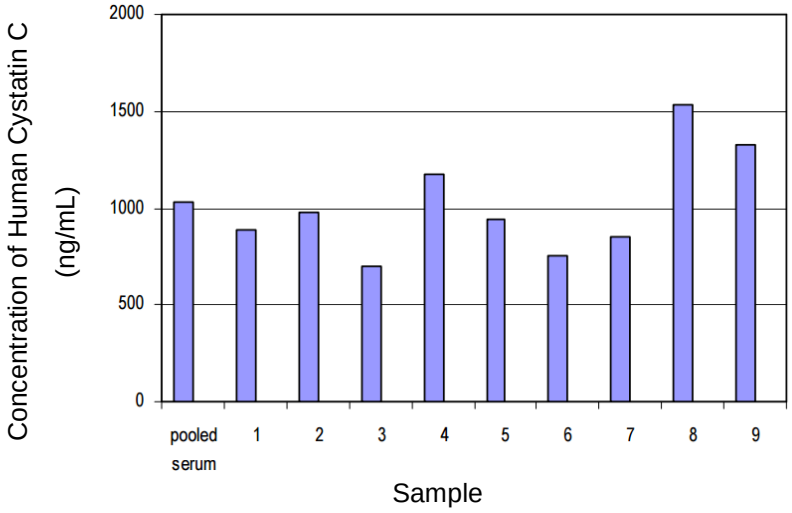


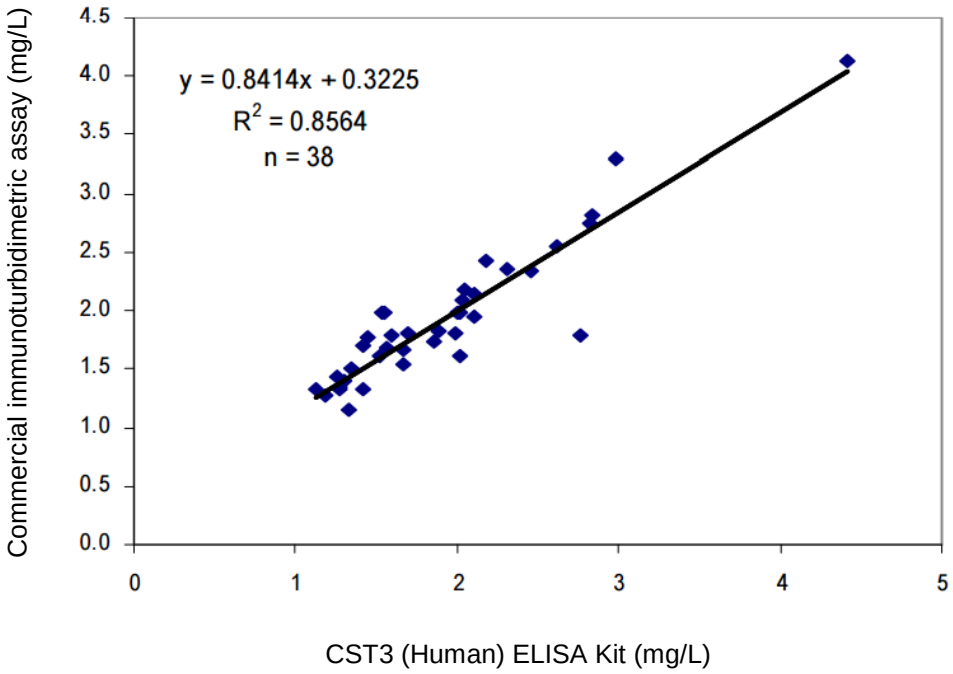
Figure 6: Samples from nine volunteers and a pooled serum were used as control sera.

✓ Reference range

The data quoted in these instructions should be used for guidance only. It is recommended that each laboratory include its own panel of control sample in the assay. Each laboratory should establish its own normal and abnormal references ranges for cystatin C levels with the assay.

✓ Method comparison

The CST3 (Human) ELISA Kit was compared to the other commercial immunoturbidimetric assay, by measuring 38 serum samples. The following correlation graph was obtained.



Resources

Troubleshooting

✓ Weak signal in all wells

Possible explanations:

- Omission of a reagent or a step
- Improper preparation or storage of a reagent
- Assay performed before reagents were allowed to come to room temperature
- Improper wavelength when reading absorbance

✓ High signal and background in all wells

Possible explanations:

- Improper or inadequate washing
- Overdeveloping; incubation time with Substrate Solution should be decreased before addition of Stop Solution
- Incubation temperature over 30°C

✓ High coefficient of variation (CV)

Possible explanation:

- Improper or inadequate washing
- Improper mixing Standards, Quality Controls or samples

References

References to cystatin C:

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Plate Layout

	1	2	3	4	5	6	7	8	9	10	11	12
A	Standard 10000	Standard 10000	Blank	Blank								
B	Standard 4000	Standard 4000	Sample 1	Sample 1								
C	Standard 2000	Standard 2000	Sample 2	Sample 2								
D	Standard 1000	Standard 1000	Sample 3	Sample 3								
E	Standard 400	Standard 400	Sample 4	Sample 4								
F	Standard 200	Standard 200	Sample 5	Sample 5								
G	QC HIGH	QC HIGH	Sample ...	Sample ...								
H	QC LOW	QC LOW	Sample ...	Sample ...								