



## **Cystatin C (human) ELISA Kit**

*Manufactured by BioVendor.*

**ALX-850-292-KI01**

**96 wells (~80 tests)**

**(Version 33: October 31, 2007)**

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**For laboratory use only. Not for human or diagnostic use.**

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**Use only the actual version of Product Data Sheet enclosed with the kit!**

## **1. Intended Use**

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The Human Cystatin C ELISA is a sandwich enzyme immunoassay for the quantitative measurement of human Cystatin C in serum, plasma, urine (see chapter 15.), cerebrospinal fluid and tissue culture medium. It is intended for research use only.

### **Features**

- The total assay time is less than 2 hours.
- The kit measures total serum Cystatin C.
- Components of the kit are provided ready-to-use and concentrated.

## **2. Storage, Expiration**

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Store the kit at 2-8°C. Under these conditions, the kit is stable until the expiration date (see label on the box).

### 3. Summary

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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-glycosylated 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.

#### **4. Test Principle**

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Surface of wells in microtitration plate is coated with polyclonal anti-human Cystatin C specific antibody. Diluted Standards, diluted Quality Controls (QC) and diluted samples are pipetted into the wells. Any human Cystatin C present is captured by immobilized antibody and unbound protein is washed away after the first incubation period. Then, a horseradish peroxidase (HRP) conjugated polyclonal anti-human Cystatin C antibody is added to the wells and incubated. Following another washing step, to remove unbound antibody-HRP conjugate, a substrate solution ( $H_2O_2$  and TMB) is added to the wells. The enzymatic reaction yields a blue product that turns yellow when acidic stop solution is added. The intensity of the colour, measured spectrophotometrically at 450 nm, is directly proportional to the amount of the human Cystatin C bound in the initial step. Concentration of unknown samples are then read off the standard curve that is constructed by plotting the absorbance values against each respective human Cystatin C standard level using a four-parameter function.

#### **5. Precautions**

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- For research use only.
- 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.
- Wear gloves, eyes and clothing protection when handling supplied immunodiagnostic material, particularly acidic Stop Solution, and Substrate Solution that contains hydrogen peroxide and tetramethylbenzidine (TMB). Avoid contact with these two latter reagents. The Stop and/or Substrate Solutions may cause skin/eye irritation. In such a case, wash the skin/eyes thoroughly with water and seek medical attention if necessary.
- All solutions supplied must not be pipetted by mouth.
- Do not drink, eat or smoke in the area where immunodiagnostic materials are being handled.
- Do not mix reagents from different kit lots.
- Reagents should not be used beyond the expiration date marked on the kit label.

## 6. Reagents Supplied

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<i>Cat. No.</i>	<i>Kit Components</i>	<i>Quantity</i>
C031111	Antibody Coated Microtiter Strips, sealed	96 wells
C032123	Conjugate Solution Concentrate (50x)	0.26 ml
C005721	Conjugate Diluent	13 ml
C033211	Human Cystatin C Standards: 10000, 4000, 2000, 1000, 400 and 200 ng/ml	0.1 ml/ vial
C034111	Quality Control High	0.1 ml
C034211	Quality Control Low	0.1 ml
C005212	Dilution Buffer Concentrate (10x)	10 ml
C006121	Wash Solution Concentrate (10x)	100 ml
C007111	Substrate Solution (TMB), ready to use	13 ml
C008111	Stop Solution (0.2 M H <sub>2</sub> SO <sub>4</sub> ), ready to use	13ml
-	Product Data Sheet + Certificate of Analysis	1 pc

## 7. Material Required but Not Supplied

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- Test tubes for diluting samples
- Precision pipettes to deliver 10-1000 µl and disposable tips
- Multichannel pipette 50-200 µl
- Microplate reader with 450 ± 10 nm filter
- Orbital microplate shaker capable of agitation at approximately 300 rpm
- Software package facilitating data generation and analysis
- Microplate washer (optional)  
[Manual washing is possible but not preferable.]
- Glassware (graduated cylinder and bottle for Wash Solution)
- Deionized (distilled) water

## 8. Preparation of Reagents

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All reagents need to be brought to room temperature prior to the assay.

Assay reagents supplied ready-to-use:

**Antibody Coated Microtiter Strips**  
**Substrate Solution (TMB)**  
**Stop Solution (0.2M H<sub>2</sub>SO<sub>4</sub>)**

Assay reagents supplied concentrated:

### **Conjugate Solution:**

Prepare the working Conjugate solution by adding 1 part Conjugate Solution Concentrate (50x) with 49 parts Conjugate Diluent. Prepare only the volume needed for the test. Mix thoroughly and gently.

Stability and storage: Conjugate Solution Concentrate is stable until the expiration date (see label on the box) if stored at 2-8°C. Do not store diluted Conjugate Solution.

### **Wash Solution:**

Dilute 100 ml of Wash Solution Concentrate (10x) with 900 ml deionized (distilled) water.

Stability and storage:

Diluted Wash Solution (1x) is stable for 1 month when stored at 2-8°C.

### **Dilution Buffer:**

Dilute 10 ml of Dilution Buffer Concentrate (10x) with 90 ml deionized (distilled) water to prepare 100 ml of Dilution Buffer (1x).

It is recommended to dilute only such a volume of Dilution Buffer expected to be used up in the one run of the test.

Stability and storage:

Working Dilution Buffer (1x) is stable for 1 week at 2-8°C.

### **Human Cystatin C Standards:**

Dilute each concentration of standard **400x** in two steps (10x and 40x) as follows: add 10 µl standard into 90 µl Dilution Buffer (dilution 10x), mix well. Use 10 µl of dilution 10x for addition into another 390 µl Dilution Buffer to prepare 400 µl of final dilution. Mix well.

Stability and storage:

Undiluted standards are stable for 9 months at 2-8°C. Do not store diluted standard solutions.

**Quality Controls:**

Dilute both, High and Low QC, at the same ratio as standards and samples, i.e. **400x**, as follows: add 10 µl QC into 90 µl Dilution Buffer (dilution 10x), mix well. Use 10 µl of dilution 10x for addition into another 390 µl Dilution Buffer to prepare final dilution. Mix well.

**Stability and storage:**

Both undiluted QC are stable for 9 months at 2-8°C. Do not store diluted QC.

**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).

If you do not use the whole plate, return unused strips in the provided aluminium bag and seal the bag carefully. Keep the unused strips at 2-8°C, protected from the moisture.

## **9. Preparation of Samples**

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**Serum/plasma samples are diluted, just prior to use in the assay, at the same ratio as standards.**

The final dilution of **400x** prepare as follows: add 10 µl sample into 90 µl Dilution Buffer (dilution 10x), mix well. Use 10 µl of dilution 10x for addition into another 390 µl Dilution Buffer to prepare final dilution. Mix well.

If you expect samples concentration beyond the calibration range (i.e. beyond 10000 – 200 ng/ml) you should adjust dilution and take into consideration a dilution factor. See Limits of Assay (chapter 12) for assistance.

(For example: if a tissue culture sample is diluted only 4x, instead of 400x, the result is divided by dilution factor 100. On the other hand, if any sample has pretty high concentration, try dilute it 800x and the result read off the calibration curve multiply by 2).

## 10. Assay Procedure

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- 1) Prepare reagents, diluted standards, controls and samples as directed in the previous sections. Remove excess microplate strips from the plate frame.
- 2) Pipet 100 µl of each diluted Standards, Quality Controls, samples and Dilution Buffer (=Blank), preferably in duplicates, into the appropriate wells.
- 3) Incubate the plate at room temperature (ca 26°C) for 30 min., shaking at about 300 rpm on the orbital microplate shaker. Incubation without shaking is the alternative that requires to extend incubation with substrate – see paragraph 9.
- 4) Wash the wells 3-times with Wash Solution (0.35 ml per well). Invert the plate and blot it against paper towels to remove the remaining Wash Solution.
- 5) Add 100 µl of Conjugate Solution into each well.
- 6) Incubate the plate at room temperature (ca 26°C) for 30 min., shaking at about 300 rpm on the orbital microplate shaker. Incubation without shaking is the alternative that requires to extend incubation with substrate – see paragraph 9.
- 7) Wash the wells 3-times with Wash Solution (0.35 ml per well). Invert the plate and blot it.
- 8) Add 100 µl of Substrate Solution. Protect from light. (Covering the plate with e.g. aluminium foil is recommended.)
- 9) Incubate the plate at room temperature for 10 minutes if the plate was shaken in previous steps. Alternatively, if the plate was not previously shaken, incubation with substrate solution is extended to 15-20 min. Do no shake with the plate during this incubation!
- 10) Stop the colour development by adding 100 µl of Stop Solution.
- 11) Determine optical density in the plate by reading absorbances at 450 nm (within 15 minutes, following the step 10).

*Note: If the microplate reader is not capable of reading absorbance greater than the absorbance of the highest standard, 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 samples. The readings at 405 nm should not replace the on-scale readings at 450 nm.*

	strip 1+2	strip 3+4	strip 5+6	strip 7+8	strip 9+10	strip 11+12
A	Standard 10000	Blank	Sample 8	Sample 16	Sample 24	Sample 32
B	Standard 4000	Sample 1	Sample 9	Sample 17	Sample 25	Sample 33
C	Standard 2000	Sample 2	Sample 10	Sample 18	Sample 26	Sample 34
D	Standard 1000	Sample 3	Sample 11	Sample 19	Sample 27	Sample 35
E	Standard 400	Sample 4	Sample 12	Sample 20	Sample 28	Sample 36
F	Standard 200	Sample 5	Sample 13	Sample 21	Sample 29	Sample 37
G	QC High	Sample 6	Sample 14	Sample 22	Sample 30	Sample 38
H	QC Low	Sample 7	Sample 15	Sample 23	Sample 31	Sample 39

Figure 1: Example of work sheet.

## 11. Calculations

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Most microtiter plate readers perform automatic calculations of analyte concentration. The standard curve is constructed by plotting the absorbance (Y) of calibrators against the *log* of the known concentration (X) of calibrators, using the four-parameter function. Alternatively, the *logit log* function can be used to linearize the calibration curve (i.e. *logit* of absorbance (Y) is plotted against *log* of the known concentration (X) of calibrators). If plotting standards concentration (X) against the respective absorbance (Y), use values of undiluted standard range: 10000, 4000, 2000, 1000, 400 and 200 ng/ml.

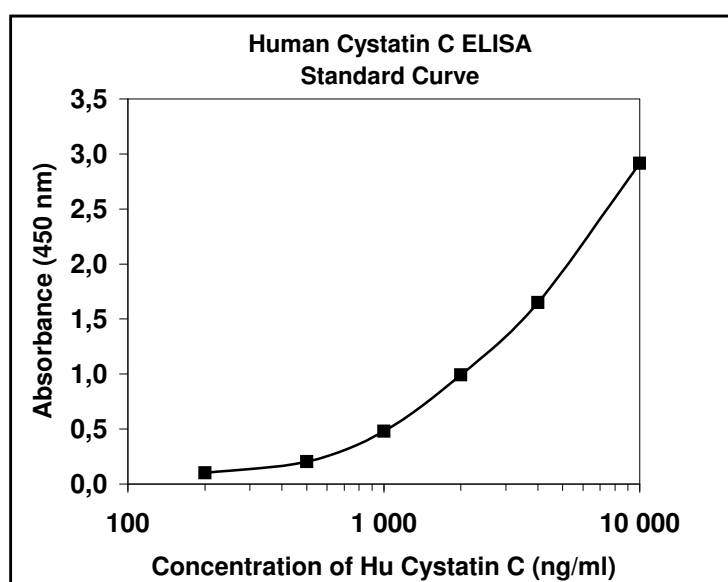


Figure 2: Standard curve for human Cystatin C is plotted using the four-parameter function as a proportion of Cystatin C concentration and absorbances at 450 nm.

Concentration of human Cystatin C in the original (undiluted) sample is directly determined from standard curve. Because the standards and samples are diluted in the same ratio (400x), any dilution factor is not used.

**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.

## **12. Limits of Assay**

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Samples exceeding Cystatin C level of 10000 ng/ml should be measured at higher dilution, e.g. **800x** and dilution factor needs to be taken into consideration. The result (read off standard curve) is then **multiplied by 2**.

Conversely: if a tissue culture sample is diluted only **4x** instead of 400x, due to lower concentration of analyte, the result (read off the standard curve) is **divided by** dilution factor **100**, 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 calibration range 10000-200 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.*

### 13. Performance Characteristics

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Typical analytical data obtained with the BioVendor Human Cystatin C ELISA are presented below.

- **Sensitivity**

The limit of detection (defined as human Cystatin C concentration giving absorbance higher than mean absorbance of blank\* plus three standard deviations of the absorbance of blank:  $A_{\text{blank}} + 3 \times \text{SD}_{\text{blank}}$ ) is defined as follows:

Analytical Limit of Detection (LOD) is calculated from the real Cystatin C values in wells and is 0.2ng/ml.

Assay Sensitivity (LOQ) takes the dilution of samples into consideration and is calculated according to the formula:

Assay Sensitivity = Analytical Limit of Detection x sample dilution = 0.2 ng/ml x 400 = 80 ng/ml.

\* Dilution Buffer is pipetted into Blank wells and the microtiter plate is blanked on air.

- **Specificity**

Sera of several mammalian species were measured in the assay. No cross-reactivity has been observed for rabbit, goat, sheep, cattle, swine and horse serum.

Some interference was, however, found in mouse and rat serum, giving the 13.3% and 22.0% signal of human serum, respectively.

- **Precision**

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

<i>Sample</i>	<i>Mean (ng/ml)</i>	<i>Standard Deviation (ng/ml)</i>	<i>CV (%)</i>
1	702	47	6.7
2	2862	144	5.0

Inter-assay (Run-to-Run, n=6)

<i>Sample</i>	<i>Mean (ng/ml)</i>	<i>Standard Deviation (ng/ml)</i>	<i>CV (%)</i>
1	600	37	6.2
2	2905	139	4.8

- **Spiking Recovery**

Serum samples were spiked to different levels of human Cystatin C, diluted with Dilution Buffer 400x, and assayed.

<i>Sample</i>	<i>Observed (ng/ml)</i>	<i>Expected (ng/ml)</i>	<i>Recovery O/E (%)</i>
1	418.05	-	-
	813.00	818.05	99.4
	1084.70	1218.05	89.1
	2103.90	2418.05	87.0
2	616.70	-	-
	931.50	1016.70	91.6
	1267.40	1416.70	89.5
	2312.90	2616.70	88.4

- **Dilution Linearity**

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

<i>Sample</i>	<i>Dilution</i>	<i>Observed (ng/ml)</i>	<i>Expected (ng/ml)</i>	<i>Recovery O/E (%)</i>
1	-	2229.60	-	-
	1:2	1168.00	1114.80	104.8
	1:4	581.40	557.40	104.3
	1:8	262.80	278.70	94.3
2	-	3107.60	-	-
	1:2	1512.80	1553.80	97.4
	1:4	728.50	776.90	93.8
	1:8	397.70	388.45	102.4

- **Effect of Sample Matrix (serum/plasma)**

Samples from 15 healthy individuals were taken and treated by different methods (serum, EDTA plasma, citrate plasma and heparin plasma), and assay results are shown below:

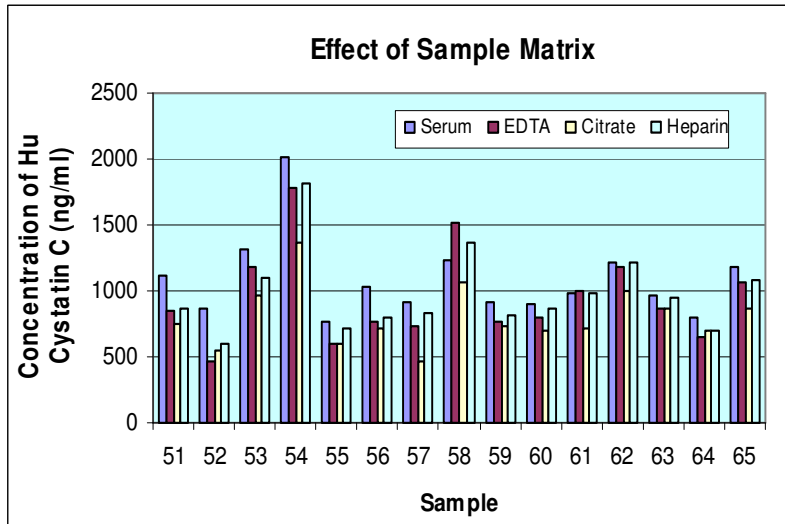


Figure 3: Fifteen serum samples were measured, each with respective EDTA, citrate and heparin plasma.

Sample Number:	Serum (ng/ml)	Plasma (ng/ml)		
		EDTA	Citrate	Heparin
51	1111	850	751	866
52	861	474	549	600
53	1316	1182	964	1105
54	2009	1784	1369	1814
55	775	606	593	721
56	1035	760	716	800
57	921	738	459	828
58	1236	1515	1059	1370
59	913	769	738	821
60	892	799	702	868
61	982	1004	710	984
62	1218	1188	996	1216
63	973	862	865	956
64	808	644	707	694
65	1179	1061	870	1082

Mean values of Cystatin C in serum, EDTA plasma, citrate plasma and heparin plasma, are set out in the following table:

Sample (n=15)	Mean (ng/ml)	Plasma/Serum (%)
Serum	1014	-
EDTA plasma	922	87.7%
Citrate plasma	789	74.2%
Heparin plasma	980	90.7%

- **Effect of freezing/thawing on the concentration of human Cystatin C in samples**  
No significant decline was observed in concentration of human Cystatin C in serum and plasma samples after repeated (5x) freezing/thawing cycles.

Sample Number:	Number of f/t Cycles	Serum (ng/ml)	Plasma (ng/ml)		
			EDTA	Citrate	Heparin
51	1x	1007	893	666	861
	3x	1249	989	660	1025
	5x	1112	966	920	1063
52	1x	696	683	660	772
	3x	699	790	660	757
	5x	667	847	642	768
53	1x	1329	1389	1092	1528
	3x	1367	1338	1158	1550
	5x	1344	1319	1254	1447
54	1x	1970	1771	1276	1891
	3x	1930	1753	1539	1963
	5x	1979	1643	1827	1994
55	1x	738	715	619	699
	3x	758	821	552	738
	5x	649	753	537	844

- **Stability of samples stored at 4 °C**

Samples should be stored at -20 °C. However, no decline in concentration of Cystatin C was observed in serum and plasma samples when stored at 4 °C for 14 days. To avoid microbial contamination, ε-aminocaproic acid and sodium azide has been added to the sample reaching final concentration of 0.3% and 0.1% concentration, respectively.

Sample Number:	Incubation: Temperature, Period	Serum (ng/ml)	Plasma (ng/ml)		
			EDTA	Citrate	Heparin
51	-20 °C	1111	850	751	866
	4 °C, 7 days	836	818	668	818
	4 °C, 14 days	856	818	751	842
52	-20 °C	861	474	549	600
	4 °C, 7 days	839	519	525	585
	4 °C, 14 days	701	524	542	528
55	-20 °C	775	606	593	721
	4 °C, 7 days	688	652	506	563
	4 °C, 14 days	681	707	588	668
56	-20 °C	1035	760	716	800
	4 °C, 7 days	996	743	723	740
	4 °C, 14 days	985	731	750	693
57	-20 °C	921	738	459	828
	4 °C, 7 days	890	688	545	781
	4 °C, 14 days	871	763	560	742

#### 14. Serum Cystatin C determination

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

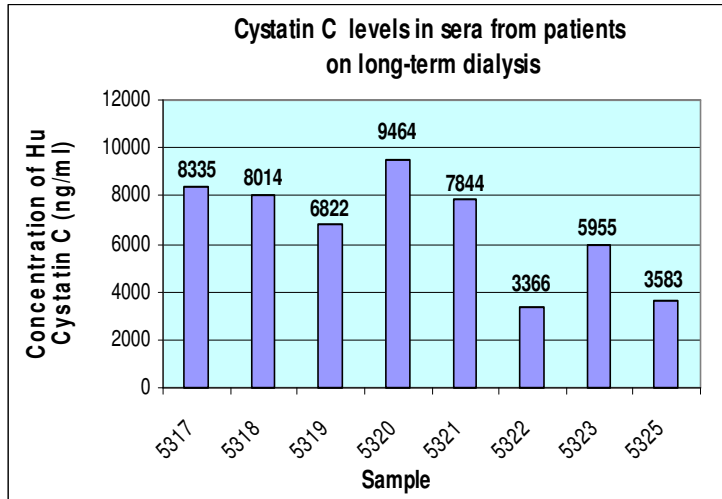


Figure 4: Cystatin C concentration was determined in serum samples from eight patients on long-term dialysis and compared to control sera. This figure corresponds with the following table:

Patients Sample Number:	Cystatin C Concentration (ng/ml)	CV (%)
5317	8335	6
5318	8014	8
5319	6822	1
5320	9464	8
5321	7844	8
5322	3366	4
5323	5955	1
5325	3583	14

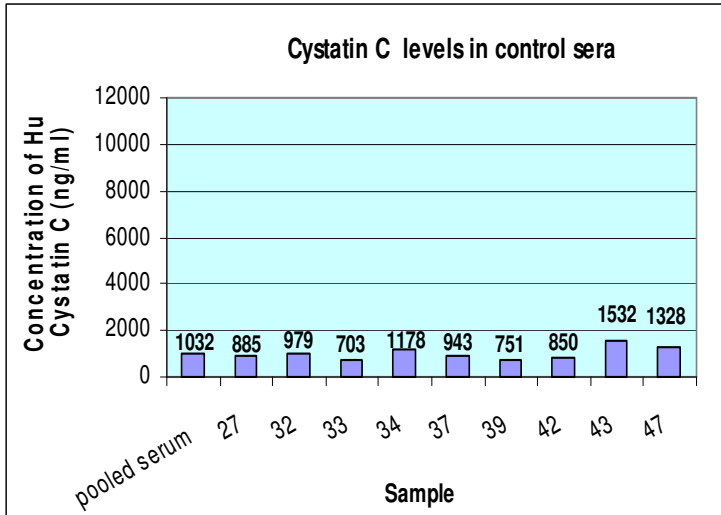


Figure 5. Samples from nine volunteers and a pooled serum were used as control sera. This figure corresponds with the following table:

Control Samples:	Cystatin C Concentration (ng/ml)	CV (%)
pooled serum	1032	11
27	885	9
32	979	4
33	703	8
34	1178	6
37	943	8
39	751	9
42	850	5
43	1532	6
47	1328	2

## 15. Urine Cystatin C determination

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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 if stored at -20°C/-70°C. Do not store 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 standard is diluted 400x, the result (read off the standard curve) has to be **devided by** dilution factor **20** in order to obtain the real concentration in the original (undiluted) sample.

- **Dilution Linearity**

Urine samples were further serially diluted with Dilution Buffer, after primary dilution 20x, and assayed.

- **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:

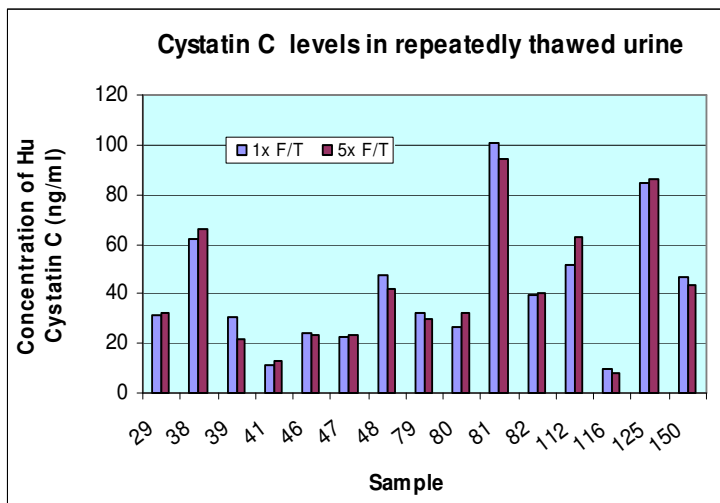


Figure 6: Cystatin C concentration was determined in urine after repeated freeze-thaw cycles. Samples were taken from fifteen individuals who were suspected to have renal dysfunction. This figure corresponds with the following table:

Patients Sample Number:	Cystatin C Concentration (ng/ml)	
	1x F/T	5x F/T
29	31	33
38	62	66
39	30	22
41	11	13
46	24	24
47	22	24
48	48	42
79	32	30
80	27	32
81	101	95
82	39	41
112	51	63
116	10	8
125	84	86
150	47	43

## 16. Method Comparison

The BioVendor Human Cystatin C ELISA was compared to the other commercial immunoassays, measuring of 116 or 112 serum samples, in immunoturbidimetry and latex nephelometric assay, respectively. The following correlation graphs were obtained:

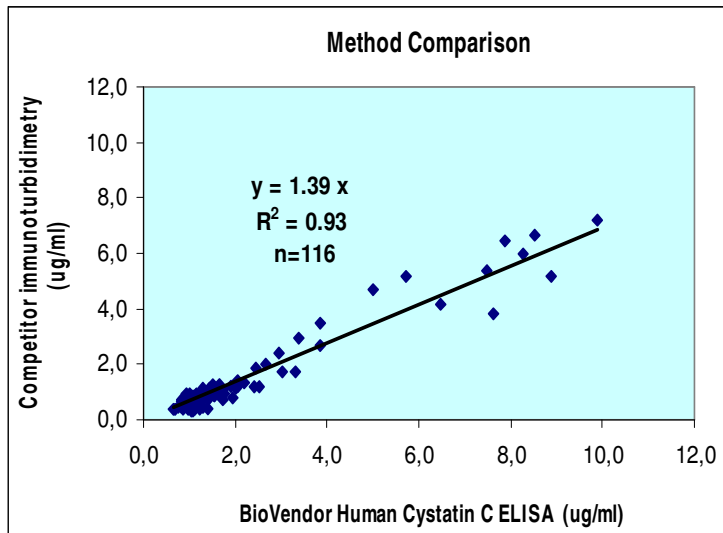


Figure 7: Cystatin C concentration was determined in serum samples from 116 healthy and non-healthy individuals. Results are expressed in units of ug/ml.

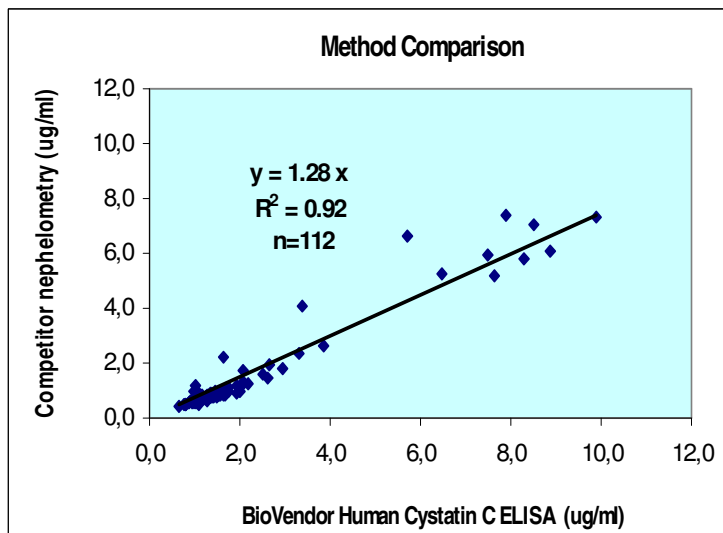


Figure 8: Cystatin C concentration was determined in serum samples from 112 healthy and non-healthy individuals. Results are expressed in units of ug/ml.

## **17. Troubleshooting and FAQs**

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### **1/ Weak signal in all wells**

Possible explanations:

- Ommiting a reagent or a step.
- Improper preparation or storage of a reagent.
- Assay performed before reagents were allowed to reach room temperature.

### **2/ High signal and background in all wells**

Possible explanations:

- Improper or inadequate washing.
- Overdeveloping; incubation time should be reduced before addition of Stop Solution.

### **3/ High coefficient of variation (CV)**

Possible explanation:

- Improper or inadequate washing.

### **4/ Why are standards diluted 400x prior to use in ELISA and what does it mean for calculation of results?**

Dilution Buffer suppresses the matrix effect of samples. It is common practice to use the same dilution for samples and standards because of simple calculation of results: concentrations of samples can be read directly off the standard curve without considering a dilution factor. Thus, in Human Cystatin C ELISA the same dilution 400x is used for Standards, Quality Controls and serum/plasma samples.

## 18. References

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### References to Cystatin C:

- Lindahl P., Abrahamson M., Bjork I.: Interaction of recombinant human cystatin C with the cysteine proteinases papain and actinidin. *Biochem J* 1992; 281: 49-55
- Ekiel I. and Abrahamson M.: Folding-related dimerization of human cystatin C. *J Biol Chem* 1996; 271: 1314-1321
- Ekiel I., Abrahamson M., Fulton D.B., Lindahl P., Storer A.C., Levadoux W., Lafrance M., Labelle S., Pomerleau Y., Groleau D., LeSauteur L., Gehring K.: NMR structural studies of human cystatin C dimmers and monomers. *J Mol Biol* 1997; 271: 266-277
- Tian S., Kusano E., Ohara T., Tabei K., Itoh Y., Kawai T., Asano Y.: Cystatin C measurement and its practical use in patients with various renal diseases. *Clin Nephrol* 1997; 48: 104-108
- Walker L.C.: Animal models of cerebral beta-amyloid angiopathy. *Brain Res Rev* 1997; 25: 70-84
- Lerner U.H., Johansson L., Ranjso M., Rosenquist J.B., Reinholt F.P., Grubb A.: Cystatin C, an inhibitor of bone resorption produced by osteoblasts. *Acta Physiol Scand* 1997; 161: 81-92
- Bokenkamp A., Domanetzki M., Zinck R., Schumann G., Byrd D., Brodehl J.: Cystatin C – a new marker of glomerular filtration rate in children independent of age and height. *Pediatrics* 1998; 101: 875-881
- Shi G.P., Sukhova G.K., Grubb A., Ducharme A., Rhode L.H., Lee R.T., Ridker P.M., Libby P., Chapman H.A.: Cystatin C deficiency in human atherosclerosis and aortic aneurysms. *J Clin Invest* 1999; 104: 1191-1197
- Risch L., Blumberg A., Huber A.: Rapid and accurate assessment of glomerular filtration rate in patients with renal transplants using serum cystatin C. *Nephrol Dial Transplant* 1999; 14: 1991-1996
- Le Bricon T., Thervet E., Benlakehal M., Bousquet B., Legendre C., Erlich D.: Changes in plasma cystatin C after renal transplantation and acute rejection in adults. *Clin Chem* 1999; 45: 2243-2249
- Cimerman N., Brguljan P.M., Krasovec M., Suskovic S., Kos J.: Twenty-four hour variations of cystatin C and total cysteine proteinase inhibitory activity in sera from healthy subjects. *Clin Chim Acta* 2000; 291: 89-95
- Stabuc B., Vrhovec L., Stabuc-Silih M., Cizej T.E.: Improved prediction of decreased creatinine clearance by serum cystatin C: use in cancer patients before and during chemotherapy. *Clin Chem* 2000; 46: 193-197
- Mangge H., Liebmann P., Tanil H., Herrmann J., Wagner C., Gallistl S., Schauenstein K., Erwa W.: Cystatin C, an early indicator for incipient renal disease in rheumatoid arthritis. *Clin Chim Acta* 2000; 300: 195-202
- Takeuchi M., Fukuda Y., Nakano I., Katano Y., Hayakawa T.: Evaluation of serum cystatin C concentrations in patients with chronic liver disease. *Eur J Gastroenterol Hepatol* 2001; 13: 951-955
- Bokenkamp A., Dieterich C., Dressler F., Muhlhaus K., Gembruch U., Bald R., Kirschstein M.: Fetal serum concentrations of cystatin C and beta2-microglobulin as predictors of postnatal kidney function. *Am J Obstet Gynecol* 2001; 185: 468-475
- Deng A., Irizarry M.C., Nitsch R.M., Growdon J.H., Rebeck G.W.: Elevation of cystatin C in susceptible neurons in Alzheimer's disease. *Am J Pathol* 2001; 159: 1061-1068
- Demirtaş S., Bozbaş A., Akbay A., Yavuz Y., Karaca L.: Diagnostics value of cystatin C for evaluation of hepatorenal syndrome. *Clin Chim Acta* 2001; 311: 81-89
- Lindholt J.S., Erlandsen E.J., Henneberg E.W.: Cystatin C deficiency is associated with the progression of small abdominal aortic aneurysms. *Br J Surg* 2001; 88: 1472-1475
- Tan G.D., Lewis A.V., James T.J., Altmann P., Taylor R.P., Levy J.C.: Clinical usefulness of cystatin C for the estimation of glomerular filtration rate in type 1 diabetes: reproducibility and accuracy compared with standard measures and iohexol clearance. *Diabetes Care* 2002; 25: 2004-2009
- Mussap M., Dalla Vestra M., Fioretto P., Saller A., Varagnolo M., Nosadini R., Plebani M.: Cystatin C is a more sensitive marker than creatinine for the estimation of GFR in type 2 diabetic patients. *Kidney Int* 2002; 61: 1453-1461
- Kazama J.J., Kutsuwada K., Ataka K., Maruyama H., Gejyo F.: Serum cystatin C reliably detects renal dysfunction in patients with various renal diseases. *Nephron* 2002; 91: 13-20
- Uchida K. and Gotoh A.: Measurement of cystatin-C and creatinine in urine. *Clin Chim Acta* 2002; 323: 121-128

- Dharnidharka V.R., Kwon C., Stevens G.: Serum cystatin C is superior to serum creatinine as a marker of kidney function: a meta-analysis. *Am J Kidney Dis* 2002; 40: 221-226
- Mares J., Stejskal D., Vavrouskova J., Urbanek K., Herzig R., Hlustik P.: Use of cystatin C determination in clinical diagnostics. *Biomed Papers* 2003; 147: 177-180
- Mojiminiyi O.A. and Abdella N.: Evaluation of cystatin C and beta-2 microglobulin as markers of renal function in patients with type 2 diabetes mellitus. *J Diabetes Complications* 2003; 17: 160-168
- Benussi L., Ghidoni R., Steinhoff T., Alberici A., Villa A., Mazzoli F., Nicosia F., Barbiero L., Broglio L., Feudatari E., Signorini S., Finckh U., Nitsch R.M., Binetti G.: Alzheimer disease-associated cystatin C variant undergoes impaired secretion. *Neurobiol Dis* 2003; 13: 15-21
- Perlemoine C., Beauvieux M.C., Rigalleau V., Baillet L., Barthes N., Derache P., Gin H.: Interest of cystatin C in screening diabetic patients for early impairment of renal function. *Metabolism* 2003; 52: 1258-1264
- Wiesli P., Schwegler B., Spinass G.A., Schmid C.: Serum cystatin C is sensitive to small changes in thyroid function. *Clin Chim Acta* 2003; 338: 87-90
- Watanabe S., Okura T., Liu J., Miyoshi K., Fukuoka T., Hiwada K., Higaki J.: Serum cystatin C level is a marker of end-organ damage in patients with essential hypertension. *Hypertens Res* 2003; 26: 895-899
- Mussap M. and Plebani M.: Biochemistry and Clinical Role of Human Cystatin C. *Crit Rev Clin Lab Sci* 2004; 41: 467-550
- Johnston N., Jernberg T., Lindahl B., Lindback J., Stridsberg M., Larsson A., Venge P., Wallentin L.: Biochemical indicators of cardiac and renal function in a healthy elderly population. *Clin Biochem* 2004; 37: 210-216
- Weiner D.E., Tighiouart H., Stark P.C., Amin M.G., MacLeod B., Griffith J.L., Salem D.N., Levey A.S., Sarnak M.J.: Kidney disease as a risk factor for recurrent cardiovascular disease and mortality. *Am J Kidney Dis* 2004; 44: 198-206
- Jernberg T., Lindahl B., James S., Larsson A., Hansson L.O., Wallentin L.: Cystatin C: a novel predictor of outcome in suspected or confirmed non-ST-elevation acute coronary syndrome. *Circulation* 2004; 110: 2342-2348
- Koenig W., Twardella D., Brenner H., Rothenbacher D.: 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* 2004
- Shlipak M.G., Sarnak M.J., Katz R., Fried L., Seliger S., Newman A., Siscovick D., Stehman-Breen C.: Cystatin-C and mortality in elderly persons with heart failure. *J Am Coll Cardiol* 2005; 45: 268-271
- Artunc F.H., Fischer I.U., Risler T., Erley C.M.: Improved estimation of GFR by serum cystatin C in patients undergoing cardiac catheterization. *Int J Cardiol* 2005; 102: 173-178
- Luc G., Bard J.M., Lesueur C., Arveiler D., Evans A., Amouyel P., Ferrieres J., Juhan-Vague I., Fruchart J.C., Ducimetiere P.: Plasma cystatin-C and development of coronary heart disease: The PRIME Study. *Atherosclerosis* 2005
- Noto D., Cefalu A.B., Barbagallo C.M., Pace A., Rizzo M., Marino G., Caldarella R., Castello A., Pernice V., Notarbartolo A., Averna M.R.: Cystatin C levels are decreased in acute myocardial infarction; Effect of cystatin C G73A gene polymorphism on plasma levels. *Int J Cardiol* 2005; 101: 213-217
- Conti M., Zater M., Lallali K., Durrbach A., Moutereau S., Manivet P., Eschwege P., Loric S.: Absence of circadian variations in urine cystatin C allows its use on urinary samples. *Clin Chem* 2005; 51: 272-273
- Seliger S.L., Longstreth W.T. Jr., Kratz R., Manolio T., Fried L.F., Shlipak M., Stehman-Breen C.O., Newman A., Sarnak M., Gillen D.L., Bleyer A., Siscovick D.S.: Cystatin C and subclinical brain infarction. *J Am Soc Nephrol.* 2005; 16: 3721-3727
- Fried L.F., Kratz R., Sarnak M.J., Shlipak M.G., Chaves P.H., Jenny N.S., Stehman-Breen C., Gillen D., Bleyer A.J., Hirsch C., Siscovick D., Newman A.B.: Kidney function as a predictor of noncardiovascular mortality. *J Am Soc Nephrol.* 2005; 16: 3728-3735
- Shlipak M.G., Wassel Fyr C.L., Chertow G.M., Harris T.B., Kritchevsky S.B., Tylavsky F.A., Satterfield S., Cummings S.R., Newman A.B., Fried L.F.: Cystatin C and mortality risk in the elderly: the health, aging, and body composition study. *J Am Soc Nephrol.* 2006; 17: 254-261
- Kato T., Heike T., Okawa K., Haruyama M., Shiraiishi K., Yoshimoto M., Nagato M., Shibata M., Kumada T., Yamanaka Y., Hattori H., Nakahata T.: A neurosphere-derived factor, cystatin C, supports differentiation of ES cells into neural stem cells. *Proc Natl Acad Sci U S A.* 2006;103: 6019-6024

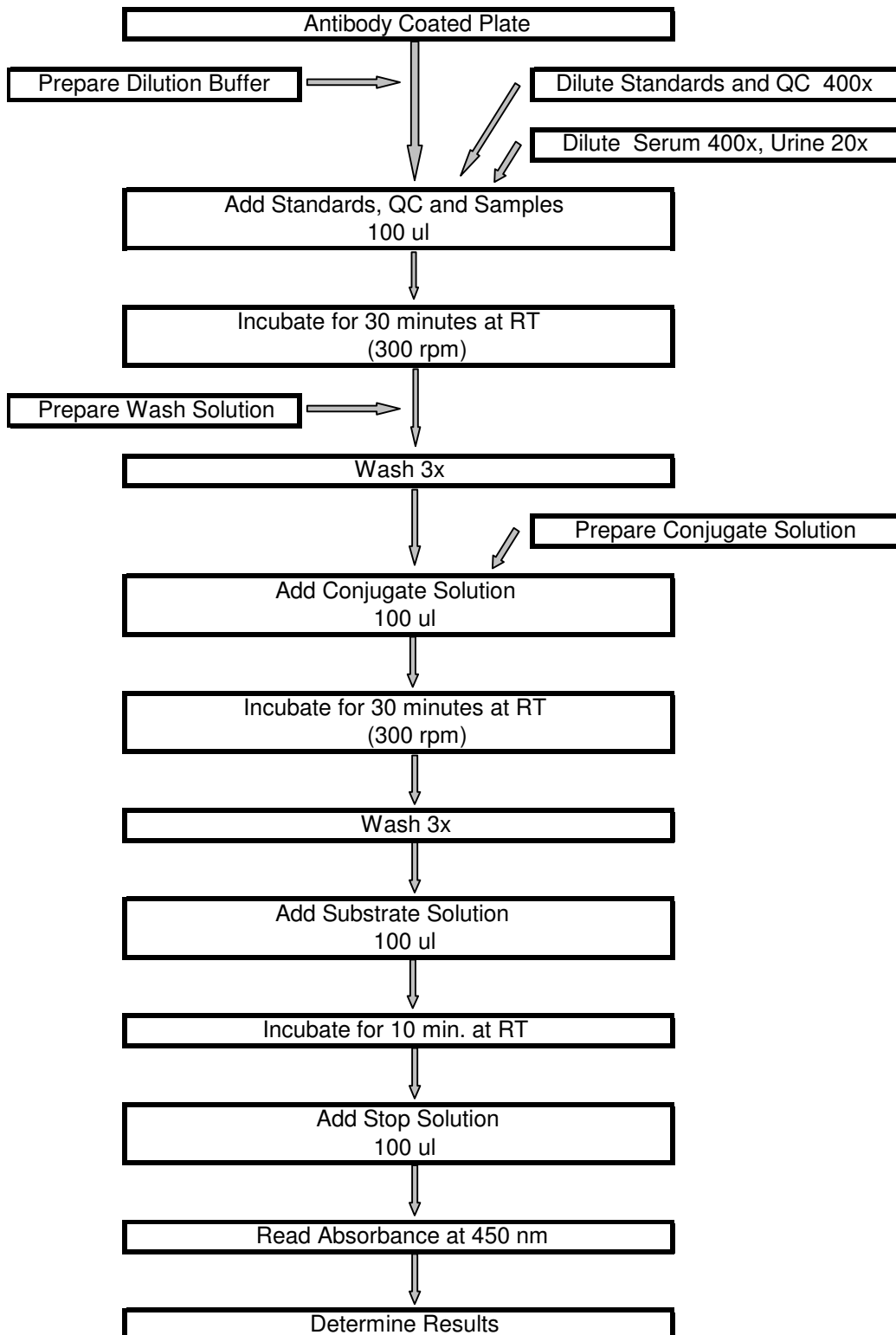
- Conti M., Moutereau S., Zater M., Lallali K., Durrbach A., Manivet P., Eschwege P., Loric S.: Urinary cystatin C as a specific marker of tubular dysfunction. *Clin Chem Lab Med*. 2006; 44: 288-291
- Macisaac R.J., Tsalamandris C., Thomas M.C., Premaratne E., Panagiotopoulos S., Smith T.J., Poon A., Jenkins M.A., Ratnaïke S.I., Power D.A. Jerums G.: Estimating glomerular filtration rate in diabetes: a comparison of cystatin-C- and creatinine-based methods. *Diabetologia*. 2006; 49: 1686-1689
- Nakashima I., Fujihara K., Fujinoki M., Kawamura T., Nishimura T., Nakamura M., Itoyama Y.: Alteration of cystatin C in the cerebrospinal fluid of multiple sclerosis. *Ann Neurol*. 2006
- Fried L.F., Biggs M.L., Shlipak M.G., Seliger S., Kestenbaum B., Stehman-Breen C., Sarnak M., Siscovick D., Harris T., Cauley J., Newman A.B., Robbins J.: Association of kidney function with incident hip fracture in older adults. *J Am Soc Nephrol*. 2007; 18: 282-286

## References to this product:

- Nedelkov D. and Nelson R.W.: Delineating protein-protein interactions via biomolecular interaction analysis-mass spectrometry. *J Mol Recognit* 2003; 16: 9-14
- Nedelkov D. and Nelson R.W.: Design and use of multi-affinity surfaces in biomolecular interaction analysis-mass spectrometry (BIA/MS): a step toward the design of SPR/MS arrays. *J Mol Recognit* 2003; 16: 15-19
- Straface E., Matarrese P., Gambardella L., Vona R., Sgadari A., Silveri M.C., Malorni W.: Oxidative imbalance and cathepsin D changes as peripheral blood biomarkers of Alzheimer disease: A pilot study. *FEBS Lett* 2005; 579: 2759-2766
- Stejskal D., Vavrouskova J., Mares J., Urbanek K.: Applications of new laboratory marker assays in neurological diagnoses - A pilot study. *Biomed Pap Med Fac Univ Palacky Olomouc Czech Repub*. 2005;149: 265-266
- Taleb S., Lacasa D., Bastard J.P., Poitou C., Canello R., Pelloux V., Viguierie N., Benis A., Zucker J.D., Bouillot J.L., Coussieu C., Basdevant A., Langin D., Clement K.: Cathepsin S, a novel biomarker of adiposity: relevance to atherogenesis. *FASEB J* 2005; 19: 1540-1542
- Barrientos L.G., Rollin P.E.: Release of cellular proteases into the acidic extracellular milieu exacerbates Ebola virus-induced cell damage. *Virology* 2006
- Liu X.D., Zeng B.F., Xu J.G., Zhu H.B., Xia Q.C.: Proteomic analysis of the cerebrospinal fluid of patients with lumbar disk herniation. *Proteomics* 2006; 6: 1019-1028
- Taleb S., Canello R., Clement K., Lacasa D.: Cathepsin S promotes human preadipocyte differentiation: possible involvement of fibronectin degradation. *Endocrinology* 2006; 147: 4950-4959
- Yang X., Wang H., Wang Z., Dong M.: Alteration and significance of serum cardiac troponin I and cystatin C in preeclampsia. *Clin Chim Acta* 2006; 374: 168-169
- Taleb S., Canello R., Poitou C., Rouault C., Sellam P., Levy P., Bouillot J.L., Coussieu C., Basdevant A., Guerre-Millo M., Lacasa D., Clement K.: Weight loss reduces adipose tissue cathepsin S and its circulating levels in morbidly obese women. *J Clin Endocrinol Metab* 2006; 91: 1042-1047

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## ASSAY PROCEDURE SUMMARY



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