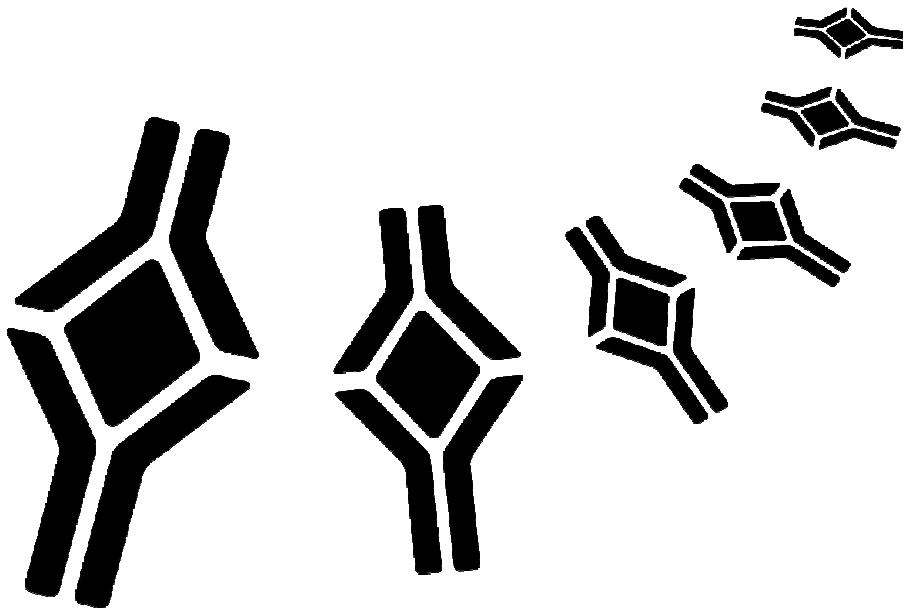


# BioVendor

Research  
and Diagnostic Products



## HUMAN CYSTATIN C ELISA

Product Data Sheet

Cat. No.: RD191009100

European  
Union:



Rest of the world:  
For research use only!

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**»» This kit is manufactured by:  
BioVendor – Laboratorní medicína a.s.**

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

## 1. INTENDED USE

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

### »» Features

- **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
- Convenient for automatization

## 2. STORAGE, EXPIRATION

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

For stability of opened reagents see Chapter 9.

### 3. INTRODUCTION

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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.

#### Areas of investigation:

Renal disease

## 4. TEST PRINCIPLE

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In the BioVendor Human Cystatin C ELISA, standards, quality controls and samples are incubated in microtiter 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.

## 5. PRECAUTIONS

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- **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

## 6. 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

## 7. REAGENT SUPPLIED

<i>Kit Components</i>	<i>State</i>	<i>Quantity</i>
Antibody Coated Microtiter Strips	ready to use	96 wells
Conjugate Solution Conc. (50x)	concentrated	0.26 ml
Conjugate Diluent	ready to use	13 ml
Set of Standards	concentrated	6 x 0.1 ml
Quality Control HIGH	concentrated	0.1 ml
Quality Control LOW	concentrated	0.1 ml
Dilution Buffer Conc. (10x)	concentrated	10 ml
Wash Solution Conc. (10x)	concentrated	100 ml
Substrate Solution	ready to use	13 ml
Stop Solution	ready to use	13 ml
Product Data Sheet + Certificate of Analysis	-	1 pc

## 8. MATERIAL REQUIRED BUT NOT SUPPLIED

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- 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 microtiter 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)

## 9. PREPARATION OF REAGENTS

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- 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 desiccant 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 months 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.**

**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 5* and *Figure 6*).



**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.

## 10. PREPARATION OF SAMPLES

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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) 1 600x 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 (1 600x). **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 Chapter 15.**

See Chapter 13 for stability of serum and plasma 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.*

## 11. ASSAY PROCEDURE

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1. Pipet **100 µl** of diluted Standards, Quality Controls, Dilution Buffer (=Blank) and samples, preferably in duplicates, into the appropriate wells. See *Figure 1* 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. Incubation without shaking is the alternative that requires to extent incubation with substrate – see paragraph 8.
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.*

	strip 1+2	strip 3+4	strip 5+6	strip 7+8	strip 9+10	strip 11+12
<b>A</b>	<b>Standard 10 000</b>	<b>Blank</b>	Sample 8	Sample 16	Sample 24	Sample 32
<b>B</b>	<b>Standard 4 000</b>	Sample 1	Sample 9	Sample 17	Sample 25	Sample 33
<b>C</b>	<b>Standard 2 000</b>	Sample 2	Sample 10	Sample 18	Sample 26	Sample 34
<b>D</b>	<b>Standard 1 000</b>	Sample 3	Sample 11	Sample 19	Sample 27	Sample 35
<b>E</b>	<b>Standard 400</b>	Sample 4	Sample 12	Sample 20	Sample 28	Sample 36
<b>F</b>	<b>Standard 200</b>	Sample 5	Sample 13	Sample 21	Sample 29	Sample 37
<b>G</b>	<b>QC HIGH</b>	Sample 6	Sample 14	Sample 22	Sample 30	Sample 38
<b>H</b>	<b>QC LOW</b>	Sample 7	Sample 15	Sample 23	Sample 31	Sample 39

Figure 1: Example of a work sheet.

## 12. CALCULATIONS

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 cystatin 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: 10 000, 4 000, 2 000, 1 000, 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.

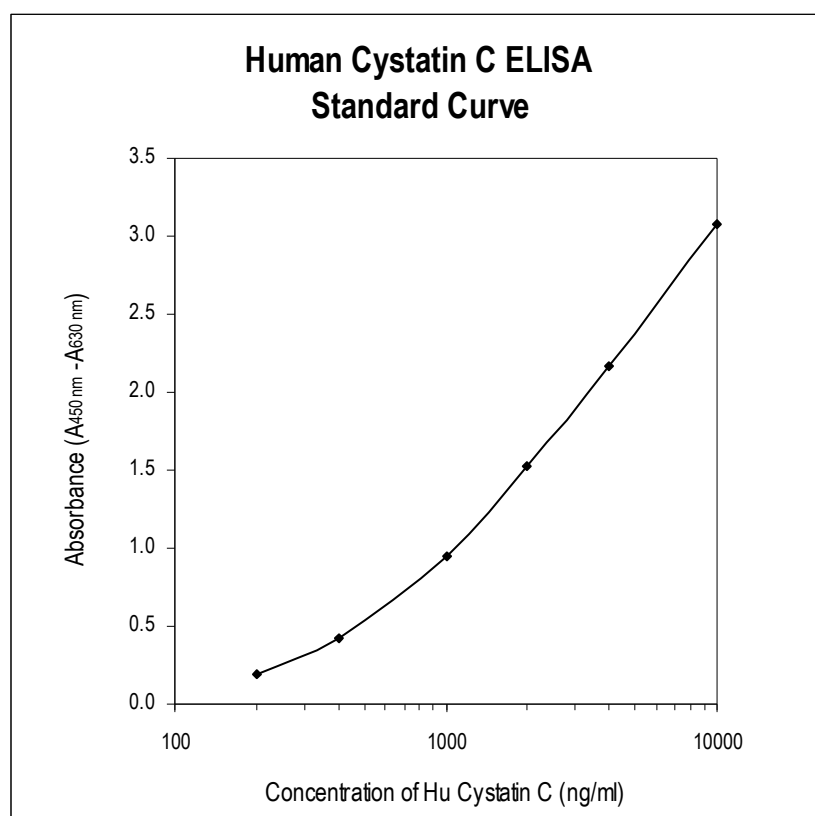


Figure 2: Typical Standard Curve for Human Cystatin C ELISA.

## 13. PERFORMANCE CHARACTERISTICS

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➤➤ Typical analytical data of BioVendor Human Cystatin C ELISA are presented in this chapter

- **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:  $A_{\text{blank}} + 3 \times \text{SD}_{\text{blank}}$ ) is calculated from the real cystatin C values in wells and is 0.2 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: 10 000, 4 000, 2 000, 1 000, 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.

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

For details please contact us at [info@biovendor.com](mailto:info@biovendor.com).

<i>Mammalian serum sample</i>	<i>Observed crossreactivity</i>
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)

<i>Sample</i>	<i>Mean (ng/ml)</i>	<i>SD (ng/ml)</i>	<i>CV (%)</i>
1	589	57	10
2	2 862	144	5

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

<i>Sample</i>	<i>Mean (ng/ml)</i>	<i>SD (ng/ml)</i>	<i>CV (%)</i>
1	600	37	6
2	2 905	139	5

- **Spiking Recovery**

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

<i>Sample</i>	<i><b>O</b>bserved (ng/ml)</i>	<i><b>E</b>xpected (ng/ml)</i>	<i>Recovery <b>O/E</b> (%)</i>
1	418	-	-
	813	818	99
	1 085	1 218	89
	2 104	2 418	87
2	617	-	-
	932	1 017	92
	1 267	1 417	90
	2 313	2 617	88

- **Linearity**

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

<i>Sample</i>	<i>Dilution</i>	<i><b>O</b>bserved (ng/ml)</i>	<i><b>E</b>xpected (ng/ml)</i>	<i>Recovery <b>O/E</b> (%)</i>
1	-	2 230	-	-
	2x	1 168	1 115	105
	4x	581	557	104
	8x	263	279	94
2	-	3 108	-	-
	2x	1 513	1 554	97
	4x	729	777	94
	8x	398	388	102



- **Effect of sample matrix**

EDTA, citrate and heparin plasmas were compared to respective serum samples from the same 15 individuals.

Results are shown below:

Volunteer No.	Serum (ng/ml)	Plasma (ng/ml)		
		EDTA	Citrate	Heparin
1	1 111	850	751	866
2	861	474	549	600
3	1 316	1 182	964	1 105
4	2 009	1 784	1 369	1 814
5	775	606	593	721
6	1 035	760	716	800
7	921	738	459	828
8	1 236	1 515	1 059	1 370
9	913	769	738	821
10	892	799	702	868
11	982	1 004	710	984
12	1 218	1 188	996	1 216
13	973	862	865	956
14	808	644	707	694
15	1 179	1 061	870	1 082
<b>Mean (ng/ml)</b>	<b>1 082</b>	<b>949</b>	<b>803</b>	<b>982</b>
<b>Mean Plasma/Serum (%)</b>	<b>-</b>	<b>87.7</b>	<b>74.2</b>	<b>90.7</b>
<b>Coefficient of determination R<sup>2</sup></b>	<b>-</b>	<b>0.82</b>	<b>0.81</b>	<b>0.87</b>

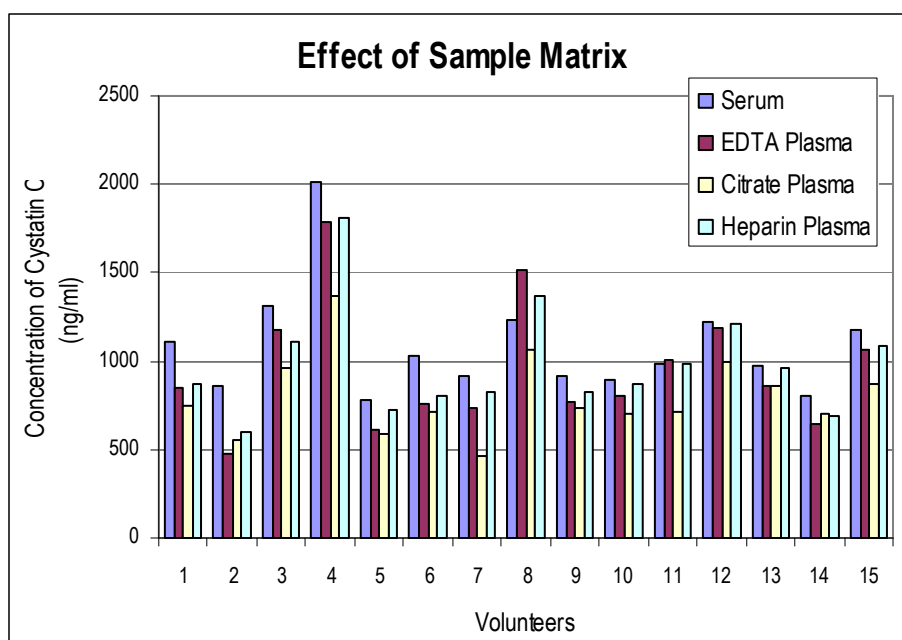


Figure 3: Cystatin C levels measured using Human Cystatin C ELISA from 15 individuals using serum, EDTA, citrate and heparin plasma, respectively.

- **Stability of samples stored at 2-8°C**

Samples should be stored at -20°C. However, no decline in concentration of cystatin C was observed in serum and plasma samples after 14 days when stored at 2-8°C. To avoid microbial contamination, samples were treated with  $\epsilon$ -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	-20°C	1 111	850	751	866
	2-8°C, 7 day	836	818	668	818
	2-8°C, 14 day	856	818	751	842
2	-20°C	861	474	549	600
	2-8°C, 7 day	839	519	525	585
	2-8°C, 14 day	701	524	542	528
3	-20°C	775	606	593	721
	2-8°C, 7 day	688	652	506	563
	2-8°C, 14 day	681	707	588	668
4	-20°C	1 035	760	716	800
	2-8°C, 7 day	996	743	723	740
	2-8°C, 14 day	985	731	750	693
5	-20°C	921	738	459	828
	2-8°C, 7 day	890	688	545	781
	2-8°C, 14 day	871	763	560	742

- **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	1 007	893	666	861
	3x	1 249	989	660	1 025
	5x	1 112	966	920	1 063
2	1x	696	683	660	772
	3x	699	790	660	757
	5x	667	847	642	768
3	1x	1 329	1 389	1 092	1 528
	3x	1 367	1 338	1 158	1 550
	5x	1 344	1 319	1 254	1 447
4	1x	1 970	1 771	1 276	1 891
	3x	1 930	1 753	1 539	1 963
	5x	1 979	1 643	1 827	1 994
5	1x	738	715	619	699
	3x	758	821	552	738
	5x	649	753	537	844

## 14. DEFINITION OF THE STANDARD

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

## 15. 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**

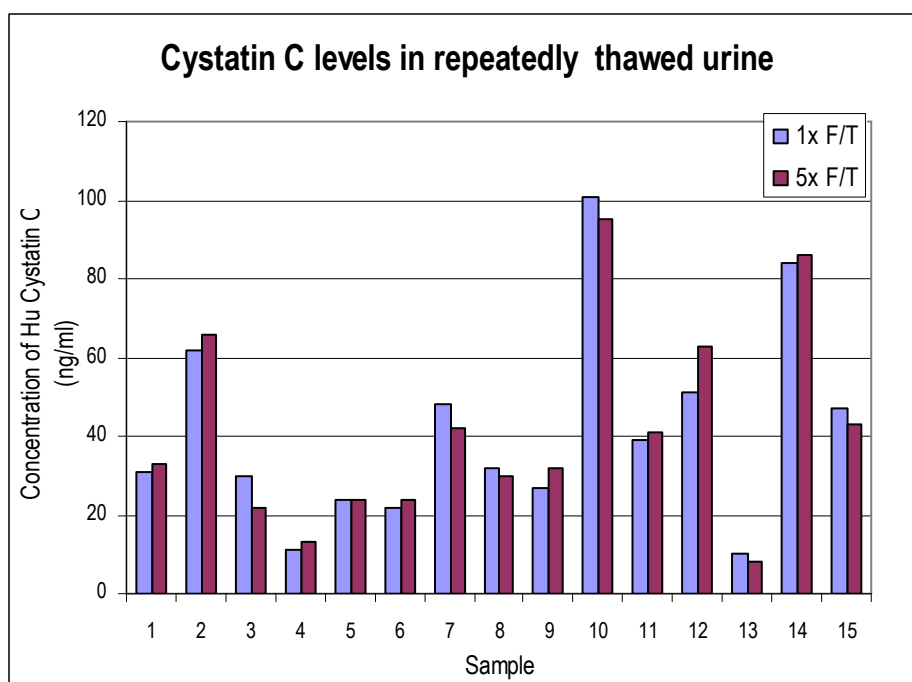
Standard curve is plotted using values of undiluted Standards: 10 000, 4 000, 2 000, 1 000, 400 and 200 ng/ml. As urine samples are diluted only **20x** whereas Standards are diluted **400x**, the result (read off the Standard curve) has to be divided by **dilution factor 20** in order to obtain the 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



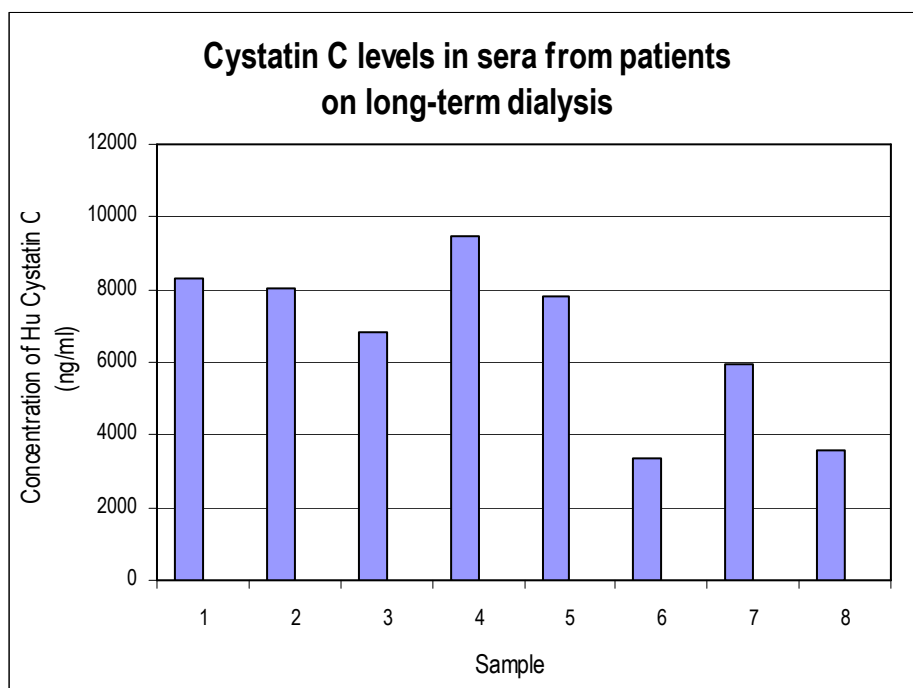
*Figure 4: Cystatin C concentration was determined in urine after repeated freeze-thaw cycles. Samples were taken from fifteen individuals who were suspected to have renal dysfunction.*

## 16. PRELIMINARY POPULATION AND CLINICAL DATA

### • 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:

Sample No.	Cystatin C (ng/ml)	CV (%)
1	8 335	6
2	8 014	8
3	6 822	1
4	9 464	8
5	7 844	8
6	3 366	4
7	5 955	1
8	3 583	14



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

Sample No.	Cystatin C (ng/ml)	CV (%)
pooled serum	1 032	11
1	885	9
2	979	4
3	703	8
4	1 178	6
5	943	8
6	751	9
7	850	5
8	1 532	6
9	1 328	2

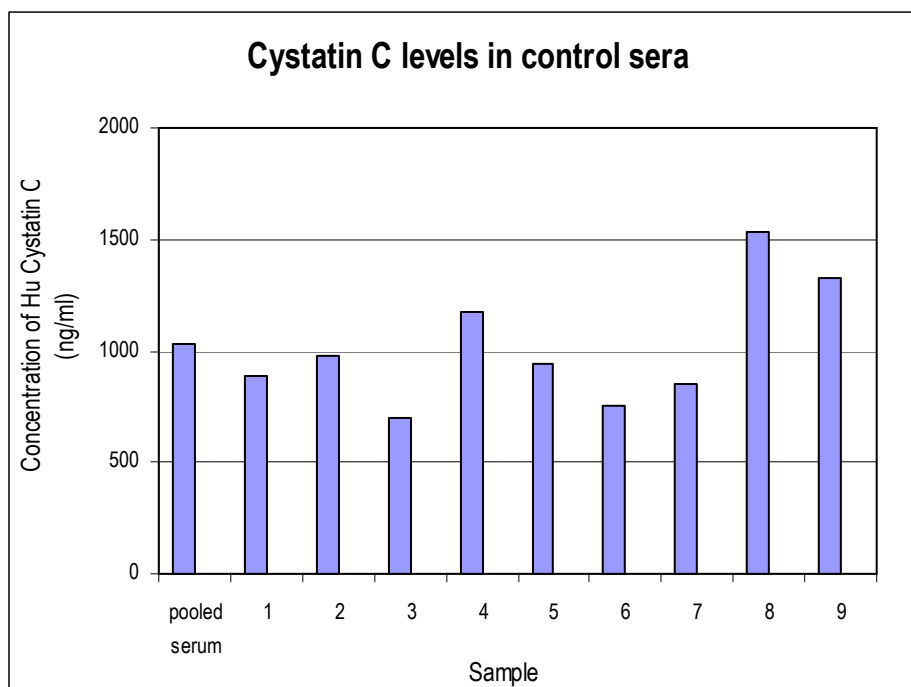


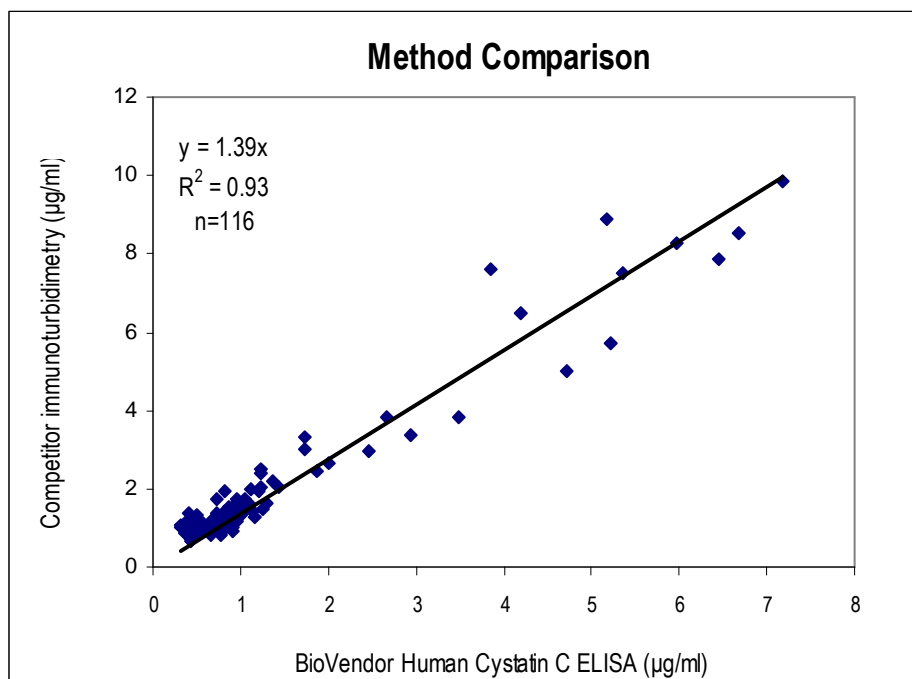
Figure 6: Samples from nine volunteers and a pooled serum were used as control sera.

- Reference range**

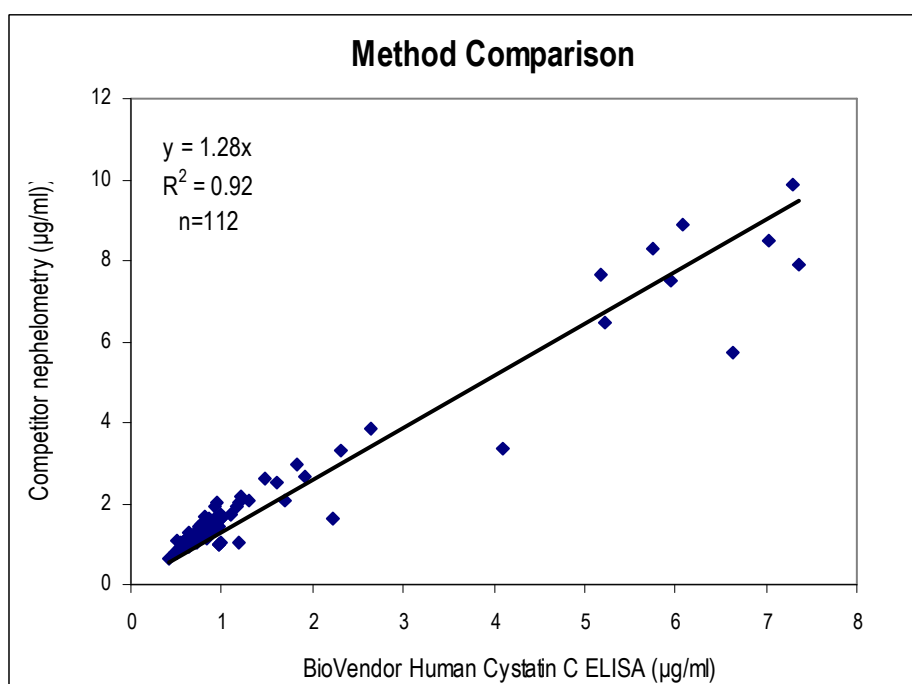
The data quoted in these instructions should be used for guidance only. It is recommended that each laboratory include its own panel of control sample in the assay. Each laboratory should establish its own normal and pathological references ranges for cystatin C levels with the assay.

## 17. METHOD COMPARISON

The BioVendor Human Cystatin C ELISA was compared to the other commercial immunoturbidimetric assay, by measuring 116 serum samples. The following correlation graph was obtained.



The BioVendor Human Cystatin C ELISA was compared to the other commercial latex nephelometric assay, by measuring 112 serum samples. The following correlation graph was obtained.





## 18. TROUBLESHOOTING AND FAQs

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### »» 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

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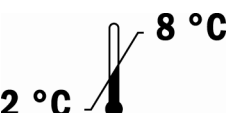


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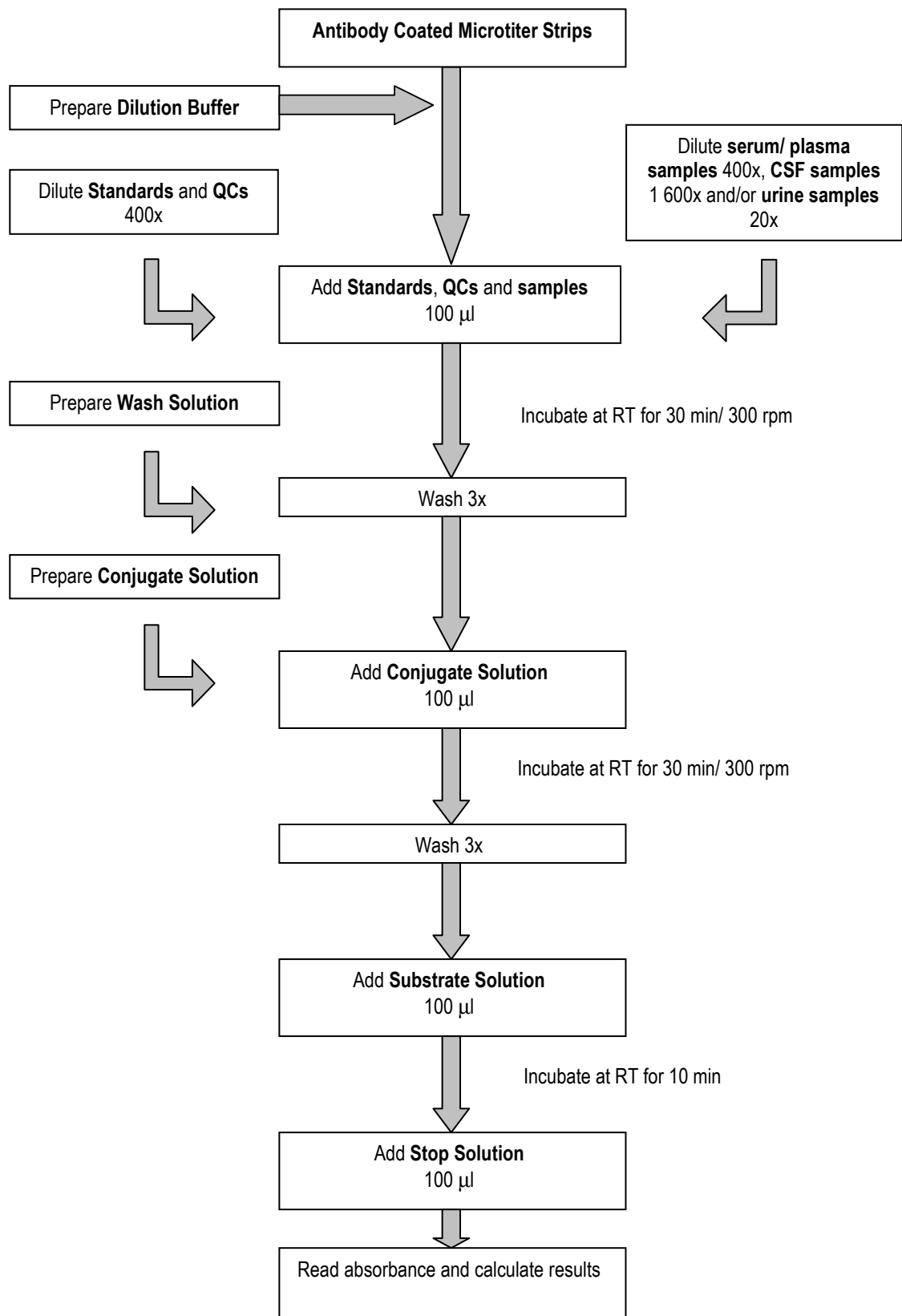
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## »» For more references on this product see our WebPages at [www.biovendor.com](http://www.biovendor.com)

## 20. EXPLANATION OF SYMBOLS

	Catalogue number
	Content
	Lot number
	See instructions for use
	Biological hazard
	Expiry date
	Storage conditions
	Identification of packaging materials
	In vitro diagnostic medical device

## Assay Procedure Summary



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