BioVendor Group

ENG

Instructions for Use:

RAT CYSTATIN C ELISA

Catalogue number:

RBL024R

For research use only!

BioVendor R&D

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CONTENT

HISTORY OF CHANGES

Previous version	Current version
	ENG.001.A
New edition	

1. INTENDED USE

Enzyme Immunoassay for the quantitative determination Cystatin C in rat serum, plasma and urine.

2. STORAGE, EXPIRATION

The kit must be stored at 2 – 8°C.
The opened components can be stored for one week at 2 – 8°C.

3. INTRODUCTION

Cystatin C or cystatin 3 (other names: gamma trace, post-gamma-globulin or neuroendocrine basic polypeptide) is a protein encoded by the CST3 gene and is synthesized in various levels by different cell-types and appears in most body fluids. Cystatins belong to a superfamily of cysteine proteases inhibitors found in both plants and animals. Cystatin C, with molecular weight of 13 260 Da and composed of 120 amino acids, lacks carbohydrate and contains two disulfide bridges located near the carboxyl terminus. Cysteine proteases play an important role in protein degradation (e.g. of photoreceptor outer segments in the retinal pigment epithelium) and the balance between these proteases and their specific inhibitors is therefore of great interest.
Cystatin C levels are increased in patients with malignant diseases, rheumatic diseases and related to the insufficiency of renal function. This protein appears to be a better marker than creatine. It may be especially useful in those cases where the creatinine measurement is not appropriate: for instance, in liver cirrhosis, in obese, in malnourished or in patients with reduced muscle mass. Cystatin C measurement may be useful in the early detection of kidney disease when other parameters might still be normal. In addition to kidney dysfunction, it has been associated with an increased risk of cardiovascular disease and heart failure in older adults.
Low levels of cystatin C indicate the breakdown of the elastic laminae and, subsequently, the atherosclerosis and abdominal aortic aneurysm. The blood level of cystatin C predicts survival after one type of heart attack. On the other hand, a high level of cystatin C in the blood after a heart attack is an ominous sign because it reflects the failure of kidney to clear cystatin C from the blood into the urine. Cystatin C was also identified, quantitated, and localized in mouse, rat, and human retinas.

4. TEST PRINCIPLE

In the Rat Cystatin C ELISA kit, standards and samples are incubated in microtitration wells pre-coated with polyclonal anti-rat Cystatin C antibody. After 60 minutes incubation followed by washing, biotin-labelled polyclonal anti-rat Cystatin C antibody is added and incubated with the captured Cystatin C for 60 minutes. After another washing, streptavidin-HRP conjugate is added. After 30 minutes incubation and the last washing step, the remaining 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 rat Cystatin C. A standard curve is constructed by plotting absorbance values against Cystatin C concentrations of standards and concentrations of unknown samples are determined using this standard curve.

5. PRECAUTIONS

1. For research use only
2. For professional laboratory use
3. The reagents with different lot numbers should not be mixed
4. To prevent cross sample contamination, use disposable labware and pipette tips
5. To protect laboratory stuff, wear protective gloves and protective clothing
6. The substrate solution should remain colourless, keep it protected from light
7. The test should be performed at standard laboratory conditions (temperature 25°C ±2°C).

6. REAGENT SUPPLIED

Item	Qty.
Antibody Coated Microtiter Plate	96 wells
Streptavidin-HRP Conjugate	13 ml
Biotin Labelled Antibody	13 ml
Master Standard (lyophilized)	2 vials
Quality Control A (lyophilized)	2 vials
Quality Control B (lyophilized)	2 vials
Dilution Buffer	2x13 ml
Wash Buffer 15x conc.	50 ml
Substrate Solution	13 ml
STOP Solution	13 ml

7. MATERIAL REQUIRED BUT NOT SUPPLIED

- Glassware and test tubes
- Microtiter plate washer
- Precision pipettes (various volumes) with tips
- Orbital shaker
- Microtiter plate reader capable of measuring absorbance at 450 nm or 450/630 nm with software for data generation

8. PREPARATION OF REAGENTS

Use new pipette tip for pipetting different reagents and samples to prevent cross-contamination. All reagents and samples should be allowed to reach the temperature 25°C ±2°C.

8.1 Preparation of Standards

Rat Cystatin C Master Standard
Refer to the Certificate of Analysis for current volume of Dilution Buffer needed for reconstitution of standard!!! Reconstitute the lyophilized Master Standard with Dilution Buffer just prior to the assay. Let it dissolve at least 15 minutes with occasional gentle shaking (not to foam). The resulting concentration of the rat Cystatin C in the stock solution is 25 ng/ml.
Prepare set of Standard solution as follows:
Use the Master Standard to produce a dilution series (as below). Mix each tube thoroughly before the next transfer. The Dilution Buffer serves as Blank.

	Volume of Standard	Dilution Buffer	Concentration
Std1	Standard 25 ng/ml (lyophilized)	See CoA	25 ng/ml
Std2	300 µl of Std1	300 µl	12.5 ng/ml
Std3	300 µl of Std2	300 µl	6.25 ng/ml
Std4	300 µl of Std3	300 µl	3.13 ng/ml
Std5	300 µl of Std4	300 µl	1.56 ng/ml
Std6	300 µl of Std5	300 µl	0.78 ng/ml
Blank	-	200 µl	0 ng/ml

8.2 Preparation of Quality Control A and B

Reconstitute the lyophilized Quality Controls in dilution buffer, for the volume information see the Certificate of Analysis. Let the QCs rehydrate for 15 min. The reconstituted Quality controls are ready to use, do not dilute them.

8.3 Preparation of Wash Buffer 1x

Prepare a working solution of Wash Buffer by adding 50 ml of Wash Buffer 15x conc. to 700 ml of deionized/ distilled water (dH_2O). Mix well. Store at 4°C for two weeks or at -20°C for long term storage.

9. PREPARATION OF SAMPLES

Rat serum, plasma or urine may be used with this assay. It is recommended to assay fresh samples. For long-term storage the serum and plasma samples should be frozen at minimum -70°C and the urine samples should be frozen in protective medium at minimum -70°C.

Recommended dilution of serum or plasma samples is 1:500. It is recommended to use the two-step dilution.
Dilution A (20x) for both singlets and duplicates: 5 µl of samples + 95 µl of Dilution Buffer.
Dilution B (25x): 5 µl of Dilution A + 120 µl of Dilution Buffer for singlets or 10 µl of Dilution A + 240 µl of Dilution Buffer for duplicates. Mix well, vortex is recommended.

Do not store the diluted samples. Do not store the diluted samples.

Recommended dilution of urine samples is 1:100. It is recommended to use the two-step dilution. Dilution A (10x) for both singlets and duplicates: 10 µl of samples + 90 µl of Dilution Buffer. Dilution B (10x): 10 µl of Dilution A + 90 µl of Dilution Buffer for singlets or 30 µl of Dilution A + 270 µl of Dilution Buffer for duplicates. Mix well, vortex is recommended.

Do not store the diluted samples. Do not store the diluted samples.

10. ASSAY PROCEDURE

1. Prepare the reagents as described in the previous chapter.
2. Pipette 100 µl of set of Standards, Quality Controls, diluted Samples and Dilution Buffer = Blank into each well. Incubate for **1 hour** at 25ºC ±2°C, shaking at 300 rpm.
3. Wash the wells 3-times with 1x Wash Buffer (350 µl/well). When finished, tap the plate against the paper towel to remove the liquid completely.
4. Pipette 100 µl of Biotin Labelled Antibody into each well. Incubate for **1 hour** at 25°C ±2°C, shaking at 300 rpm.
5. Wash the wells as described in point 3.
6. Pipette 100 µl of HRP-labelled Antibody Conjugate into each well. Incubate for **30 minutes** at 25°C ±2°C, shaking at 300 rpm.
7. Wash the wells as described in point 3.
8. Pipette 100 µl Substrate solution, incubate for **10 min,** at 25°C ±2°C**.** Avoid exposure to the light during this step.
9. Pipette 100 µl of STOP solution.

Read the signal at 450 or 450/630 nm within 15 min.

11. PERFORMANCE CHARACTERISTICS

Samples used in the tests were diluted 1:500 as recommended and assayed. The results are multiplied by the dilution factor.

11.1 Sensitivity

The limit of detection, defined as a concentration of canine Cystatin C giving absorbance higher than absorbance of blank + 3 standard deviations, is better than 0,009 ng/ml of sample.

11.2 Precision

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

Sample	Mean (ng/ml)	SD	CV (%)
1	1766	88	5.3
2	1768	77	4.3

Inter-assay (Run-to-run) (n=8)

Sample	Mean (ng/ml)	SD	CV (%)
1	1690	103	6.0
2	1858	100	5.4

11.3 Accuracy

11.3.1 Dilution linearity

Sample	Dilution	Measured concentration (ng/ml)	Expected concentration (ng/ml)	Yield (%)
Serum 1		2041	-	-
	2x	1031	1021	101
	4x	594	510	116
	8x	283	255	111
Serum 2		2842	-	-
	2x	1420	1421	100
	4x	690	711	97
	8x	324	355	91.3

Sample	Dilution	Measured concentration (ng/ml)	Expected concentration (ng/ml)	Yield (%)
Urine 1		734	-	-
	2x	372	367	107
	4x	176	184	96
	8x	86	92	93

11.3.2 Spiking Recovery

Sample	Measured concentration (ng/ml)	Expected concentration (ng/ml)	Yield (%)
Serum 1	1028	-	-
	3735	3528	106
	2343	2278	103
	1695	1653	103
Serum 2	1773	-	-
	4558	4273	107
	3188	3023	106
	2513	2398	105

Sample	Measured concentration (ng/ml)	Expected concentration (ng/ml)	Yield (%)
Urine 1	538	-	-
	766	788	97
	953	1038	92
	1370	1538	89

12. DEFINITION OF THE STANDARD

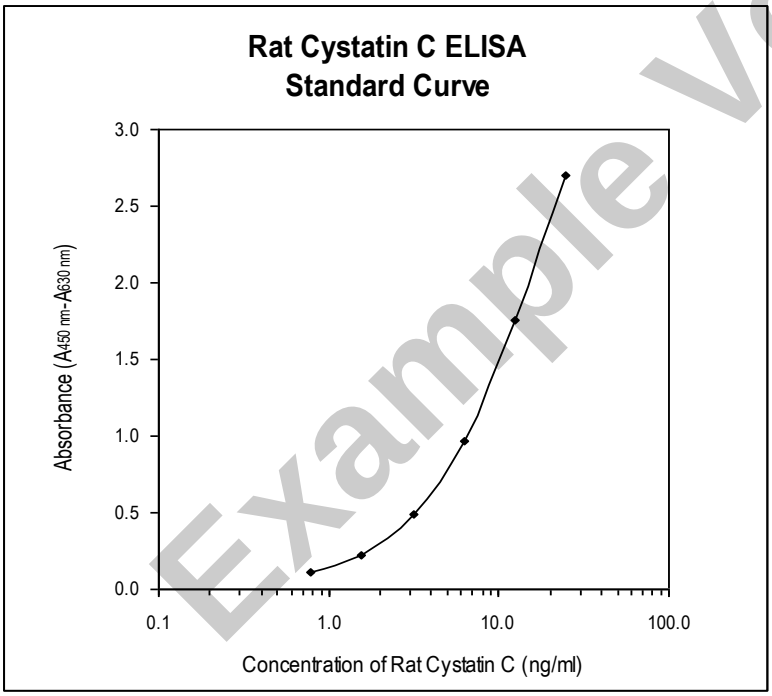
The recombinant rat Cystatin C is used as the Standard. The recombinant rat Cystatin C, produced in E.coli, is 14.93 kDa protein..

13. REFERENCE RANGE

It is recommended that each laboratory include its own panel of control samples in the assay. Each laboratory should establish its own normal and pathological reference ranges for rat Cystatin C levels with the assay.

14. CALCULATION

The standard curve needs to be measured in every test. Most of the microplate reader can automatically calculate the analyte concentration using 4-parameter algorithm or alternative functions to fit the standard points properly. The concentrations need to be multiplied by the dilution factor, either automatically by reader or manually.



15. REFERENCES

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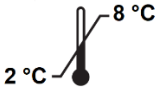
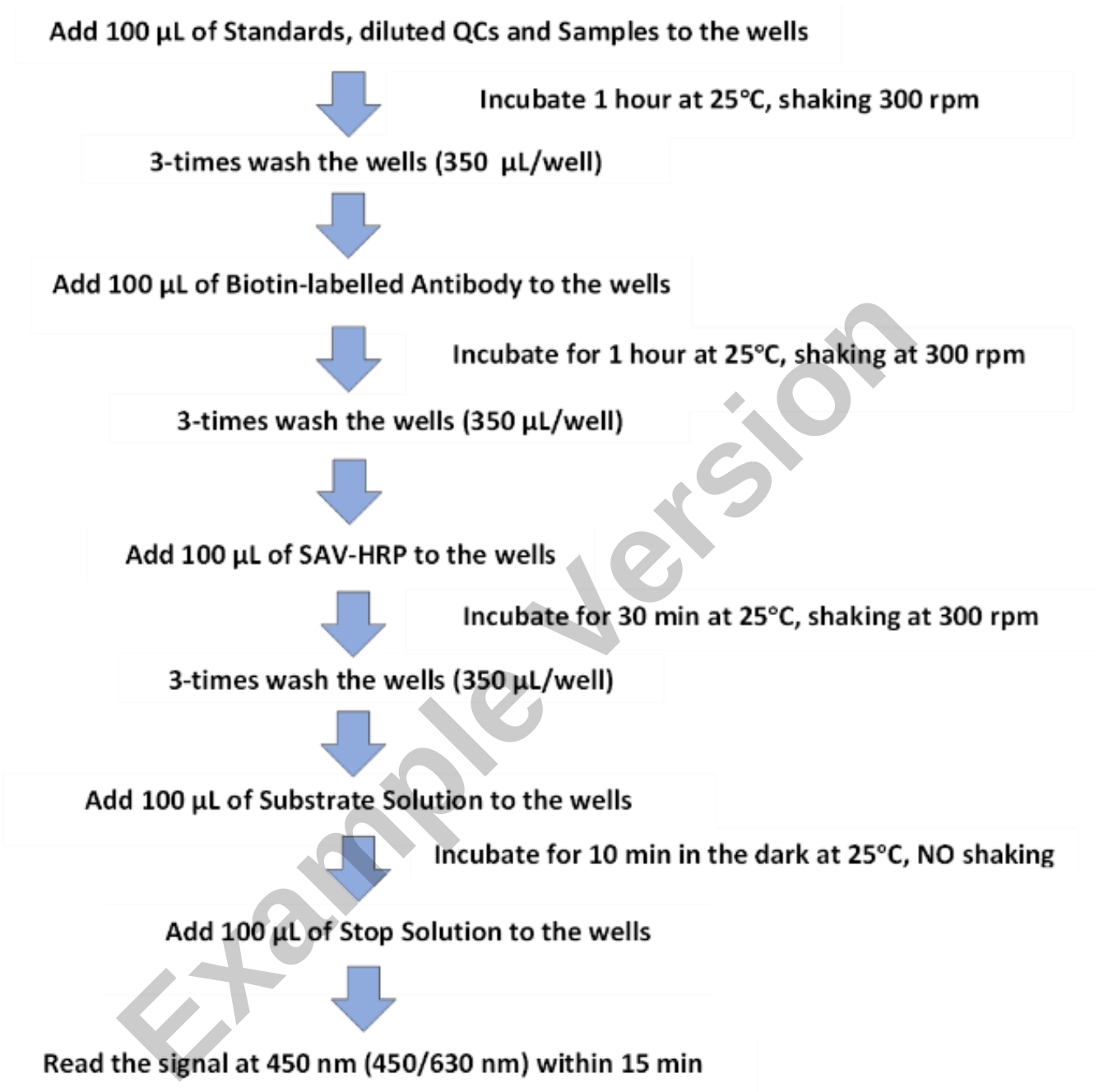
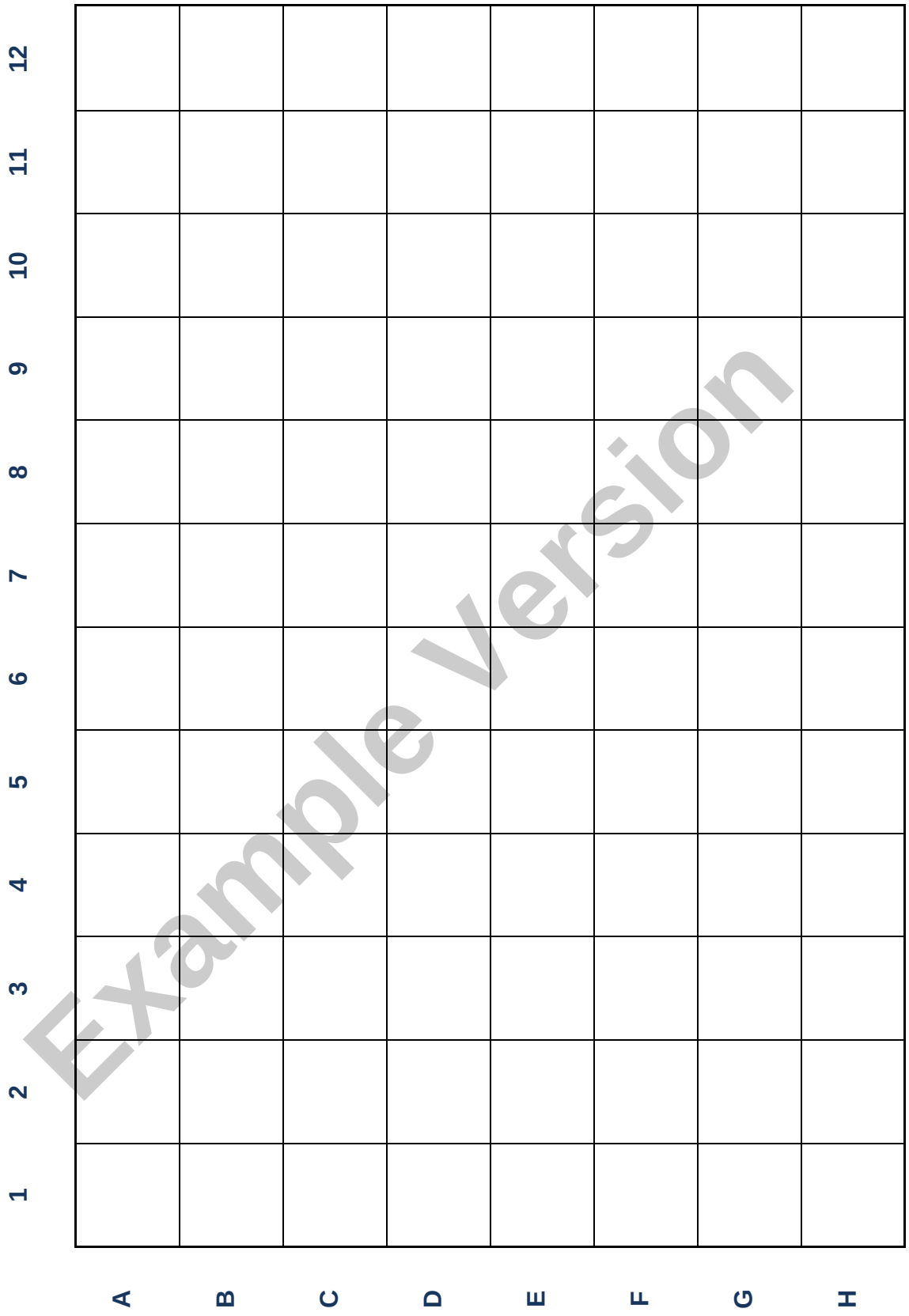
16. EXPLANATION OF SYMBOLS

	Catalogue number
	Batch code
	Caution
	Use by date
	Temperature limit
	Manufacturer
	Read electronic instructions for use - eIFU
	The content is sufficient for 96 tests
	Biological risks

17. ASSAY PROCEDURE - SUMMARY





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