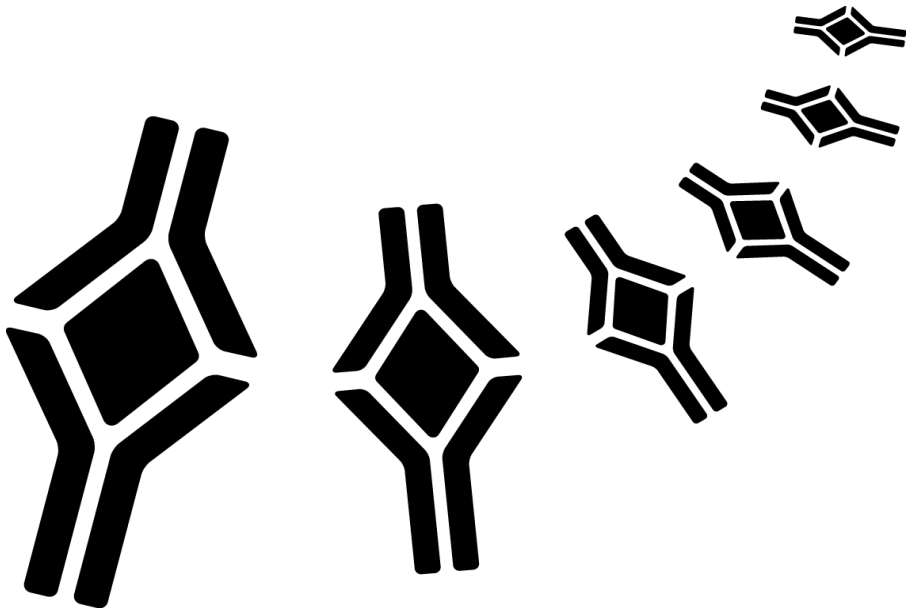
BioVendor
Research
and Diagnostic Products



CANINE CYSTATIN C ELISA

Product Data Sheet

Cat. No.: RD491009100R

For Research Use Only

CONTENTS

» **This kit is manufactured by:**
BioVendor – Laboratorni medicina a.s.

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

1. INTENDED USE

The RD491009100R Canine Cystatin C ELISA is a sandwich enzyme immunoassay for the quantitative measurement of canine cystatin C.

Features

- **It is intended for research use only**
- The total assay time is less than 3 hours
- The kit measures total cystatin C in canine serum and urine
- Assay format is 96 wells
- Quality Controls are animal serum based. No human sera are used
- Standard is recombinant protein based
- Components of the kit are provided ready to use, concentrated or lyophilized

2. STORAGE, EXPIRATION

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

Cystatin C is a non-glycosylated basic protein belonging to the super-family of cysteine proteinase inhibitors. It consists of a single polypeptide chain having 120 amino acids.
It is produced by all nucleated cells within the body and is released during phagocytosis and inflammation. In the kidney, cystatin C is freely filtrated through the glomerulus and reabsorbed and catabolized in the proximal renal tubules. The rate of cystatin C synthesis is constant, independent of age, gender and muscle mass. High concentrations can be found in serum, seminal fluid, cerebrospinal fluid (CSF), and synovial fluid, and lower concentrations can be found in urine.
In human medicine, cystatin C is the most important endogenous serum marker of renal function assessment. Cystatin C evaluation is able to detect an earlier stage of decreased glomerular filtration rate (GFR) than other parameters (serum creatinine, creatinine clearanced etc.) and it is considered particularly useful in patients with a high risk of developing nephropathies. Imbalance between cystatin C and cysteine proteinases is associated with inflammation, cancer, Alzheimer's disease, multiple sclerosis and hereditary cystatin C amyloid angiopathy. An increased level has been found in patients with autoimmune diseases. On the other hand, low concentration of cystatin C presents a risk factor for secondary cardiovascular events.
In veterinary medicine, there are multiple reports of the use of cystatin C in the evaluation of renal function indicating that cystatin C is also the most important serum (urine) marker of renal function assessment in dogs.

Areas of investigation:
Renal and kidney disease

4. TEST PRINCIPLE

In the BioVendor Canine Cystatin C ELISA, standards, quality controls and samples are incubated in microtiter plate wells pre-coated with polyclonal anti-canine cystatin C antibody. After 60 minutes incubation and washing, polyclonal anti-canine cystatin C antibody, conjugated with horseradish peroxidase (HRP) is added to the wells and incubated for 60 minutes with the 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

- **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 animal origin. These materials should be handled as potentially infectious
- 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

Kit Components	*State*	*Quantity*
Antibody Coated Microtiter Strips	ready to use	96 wells
Conjugate Solution Conc. (50x)	concentrated	0.3 ml
Conjugate Diluent	ready to use	13 ml
Master Standard	lyophilized	2 vials
Quality Control HIGH	lyophilized	2 vials
Quality Control LOW	lyophilized	2 vials
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

- Deionized (distilled) water
- Test tubes for diluting samples
- Glassware (graduated cylinder and bottle) for Wash Solution (Dilution Buffer)
- Precision pipettes to deliver 2–1 000 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

» **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 or lyophilized:**

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.

Canine Cystatin C Master Standard
Refer to Certificate of Analysis for current volume of distilled water needed for reconstitution of standard!!!
Reconstitute the lyophilized Master Standards with distilled water just prior to the assay. Let it dissolve at least 15 minutes with occasionally gently shaking (not to foam). The resulting concentration of the canine cystatin C in the stock solution is **10 ng/ml**.

Prepare set of standards using Dilution Buffer as follows:

Volume of Standard	*Dilution Buffer*	*Concentration*
Stock	-	10 ng/ml
300 µl of stock	300 µl	5 ng/ml
300 µl of 5 ng/ml	300 µl	2.5 ng/ml
300 µl of 2.5 ng/ml	300 µl	1.25 ng/ml
300 µl of 1.25 ng/ml	300 µl	0.625 ng/ml
300 µl of 0.625 ng/ml	300 µl	0.31 ng/ml

Prepared Standards are ready to use, do not dilute them.
Stability and storage:
The reconstituted standard stock solution must be used immediately.
Do not store the diluted Standard solutions.

Quality Controls HIGH, LOW
Refer to the Certificate of Analysis for current volume of Dilution Buffer needed for reconstitution and for current Quality Control concentration!!!
Reconstitute each Quality Control (HIGH and LOW) with Dilution Buffer just prior to the assay. Let it dissolve at least 15 minutes with occasional gentle shaking (not to foam).
The reconstituted Quality Controls are ready to use, do not dilute them.
Stability and storage:
The reconstituted Quality Controls must be used immediately. Avoid repeated freeze/thaw cycles.
Do not store the reconstituted Quality Controls.
Note:
Concentration of analyte in Quality Controls need not be anyhow associated with normal and/or pathological 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.

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

The kit measures cystatin C in serum and urine.

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.

Serum samples:
Dilute samples 1 000x with Dilution Buffer just prior to the assay in two steps as follows:
Dilution A (20x):
Add 5 µl of sample into 95 µl of Dilution Buffer. **Mix well** (not to foam). Vortex is recommended.
Dilution B (50x):
Add 10 µl of Dilution A into 490 µl of Dilution Buffer for duplicates to prepare final dilution (1 000x). **Mix well** (not to foam). Vortex is recommended.

Urine samples:
Dilute samples (urine) 100x with the Dilution Buffer just prior to the assay, a.g. add 5 µl of sample into 495 µl of Dilution Buffer. **Mix well** (not to foam). Vortex is recommended.

Dilute urine samples (dog patients with serum creatinine > 1.4 mg/dl) at least 1 000x with Dilution Buffer in two steps as follows:
Dilution A (20x):
Add 5 µl of sample into 95 µl of Dilution Buffer. **Mix well** (not to foam). Vortex is recommended.
Dilution B (50x):
Add 10 µl of Dilution A into 490 µl of Dilution Buffer for duplicates to prepare final dilution (1 000x). **Mix well** (not to foam). Vortex is recommended.

Stability and storage:
Samples should be stored at -20°C, or preferably at -80°C for long-term storage. Avoid repeated freeze/ thaw cycles.
Do not store the diluted samples.

See Chapter 13 for stability of serum samples when stored at 2–8°C, effect of freezing/thawing 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

1. Pipet **100 µl** of Standards, Quality Controls, Blank (Dilution Buffer) 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 **60 minutes**, shaking at ca. 300 rpm on an orbital microplate shaker.
3. Wash the wells 5-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 **60 minutes**, shaking at ca. 300 rpm on an orbital microplate shaker.
6. Wash the wells 5-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 **15 minutes** at room temperature. The incubation time may be extended, 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 absorbance 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 three times. 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
A	**Standard 10**	**Blank**	Sample 8	Sample 16	Sample 24	Sample 32
B	**Standard 5**	Sample 1	Sample 9	Sample 17	Sample 25	Sample 33
C	**Standard 2.5**	Sample 2	Sample 10	Sample 18	Sample 26	Sample 34
D	**Standard 1.25**	Sample 3	Sample 11	Sample 19	Sample 27	Sample 35
E	**Standard 0.625**	Sample 4	Sample 12	Sample 20	Sample 28	Sample 36
F	**Standard 0.31**	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 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.

The measured concentration of samples calculated from the standard curve must be multiplied by their respective dilution factor, because samples have been diluted prior to the assay, e.g. 2.5 ng/ml (from standard curve) x 1 000 (dilution factor) = 2 500 ng/ml.

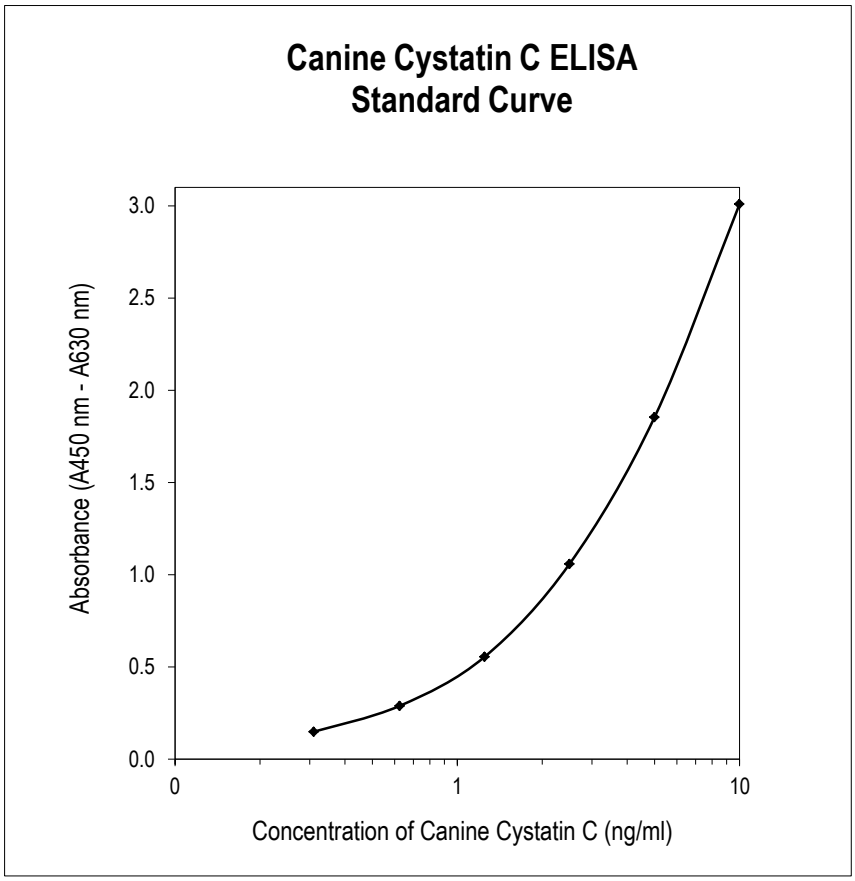


Figure 2: Typical Standard Curve for Canine Cystatin C ELISA.

13. PERFORMANCE CHARACTERISTICS

Typical analytical data of BioVendor Cystatin 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: $A_{blank} + 3xSD_{blank}$ is calculated from the real cystatin C values in wells and is 0.005 ng/ml.
*Dilution Buffer is pipetted into blank wells.

- **Limit of assay**

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

- **Specificity**

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

Sera of several mammalian species and human serum were measured in the assay. See results below. For details please contact us at info@biovendor.com.

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

» **Presented results are multiplied by respective dilution factor**

- **Precision**

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

Sample	*Mean (µg/ml)*	*SD (µg/ml)*	*CV (%)*
Serum 1	0.68	0.06	8.41
Serum 2	4.60	0.19	4.20

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

Sample	*Mean (µg/ml)*	*SD (µg/ml)*	*CV (%)*
Serum 1	0.69	0.03	4.12
Serum 2	4.42	0.32	7.28

- **Spiking Recovery**

Samples were spiked with different amounts of canine cystatin C and assayed.

Sample	***O**bserved (µg/ml)*	***E**xpected (µg/ml)*	*Recovery **O/E** (%)*
Serum 1	1.52	-	-
	2.10	2.02	104.2
	2.62	2.52	104.2
	3.63	3.52	103.2
Serum 2	1.05	-	-
	1.66	1.55	107.7
	2.11	2.05	103.2
	3.16	3.05	103.6
Urine 1	0.51	-	-
	0.60	0.57	105.6
	0.63	0.63	100.4
	0.79	0.76	104.3
Urine 2	0.16	-	-
	0.22	0.22	98.3
	0.30	0.28	107.3
	0.42	0.41	102.1

- **Linearity**

Samples were serially diluted with Dilution Buffer and assayed.

Sample	*Dilution*	***O**bserved (µg/ml)*	***E**xpected (µg/ml)*	*Recovery **O/E** (%)*
Serum 1	-	2.88	-	-
	2x	1.36	1.44	94.5
	4x	0.64	0.72	88.2
	8x	0.31	0.36	86.0
Serum 2	-	7.77	-	-
	2x	3.60	3.89	92.6
	4x	1.71	1.94	87.9
	8x	0.86	0.97	88.3
Urine 1	-	0.74	-	-
	2x	0.36	0.37	96.4
	4x	0.17	0.18	92.1
	8x	0.09	0.09	92.7
Urine 2	-	12.55	-	-
	2x	5.97	6.28	95.2
	4x	2.88	3.14	91.7
	8x	1.42	1.57	90.7

- **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 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 (µg/ml)*
1	-80°C	1.61
	2–8°C, 1 day	1.58
	2–8°C, 7 day	1.64
2	-80°C	9.69
	2–8°C, 1 day	10.14
	2–8°C, 7 day	9.97
3	-80°C	1.87
	2–8°C, 1 day	1.87
	2–8°C, 7 day	1.87

- **Effect of Freezing/Thawing**

No decline was observed in concentration of canine cystatin C in serum and urine samples after repeated freeze/thaw cycles. However it is recommended to avoid unnecessary repeated freezing/thawing of the samples. All the tested urine samples were stabilized using Stabilization Solution for Urine Samples (CS019)

Sample	*Number of f/t cycles*	*Serum (µg/ml)*
1	1x	1.95
	3x	1.86
	5x	1.84
2	1x	10.55
	3x	10.76
	5x	10.56
3	1x	2.16
	3x	2.22
	5x	1.98

Sample	*Number of f/t cycles*	*Urine (µg/ml)*
1	1x	0.74
	2x	0.72
	3x	0.71
2	1x	0.51
	2x	0.50
	3x	0.50
3	1x	0.16
	2x	0.16
	3x	0.15

14. DEFINITION OF THE STANDARD

In this assay as the Standard the recombinant canine cystatin C is used. This cystatin C protein composed from 133 amino acid residues was produced in *E.coli* system. The apparent molecular weight is 14.85 kDa.

15. CLINICAL DATA

The following results were obtained when serum and urine dog samples with various serum creatinine concentrations were assayed with the Biovendor Canine Cystatin C ELISA at the Department of Animal Medicine and Surgery, Veterinary School, University of Murcia, Spain (kindly provided by Prof. J.J. Ceron).

Group of samples	*Serum creatinine (mg/dl)*	*Serum Cystatin C: Mean (MIN; MAX) (µg/ml)*	*Urine Cystatin C: Mean (MIN; MAX) (µg/ml)*
A (n=8)	≤ 1.4	1.23 (0.67; 1.97)	0.11 (0.05; 0.20)
B (n=6)	> 5.0	6.44 (4.91; 12.60)	21.08 (13.18; 31.65)

16. METHOD COMPARISON

BioVendor Canine Cystatin C ELISA has not been compared to any other immunoassay.

17. TROUBLESHOOTING AND FAQS

›› 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

18. REFERENCES

›› References to canine cystatin C:

- Monti P, Benchekroun G, Berlato D, Archer J: Initial evaluation of canine urinary cystatin C as a marker of renal tubular function. *J Small Anim Pract*; **53**(5):254-259 (2012)
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- Almy FS, Christopher MM, King DP, Brown SA: Evaluation of cystatin C as an endogenous marker of glomerular filtration rate in dogs. *J Vet Intern Med*; **16**(1):45-51 (2002)
- Coll E, Botey A, Alvarez L, Poch E, Quinto L, Saurina A, Vera M, Piera C, Darnell A: Serum Cystatin C as a New Marker for Noninvasive Estimation of Glomerular Filtration Rate and as a Marker for Early Renal Impairment. *Am J Kidney Dis*; **36**(1):29-34 (2000)

›› References to this product:

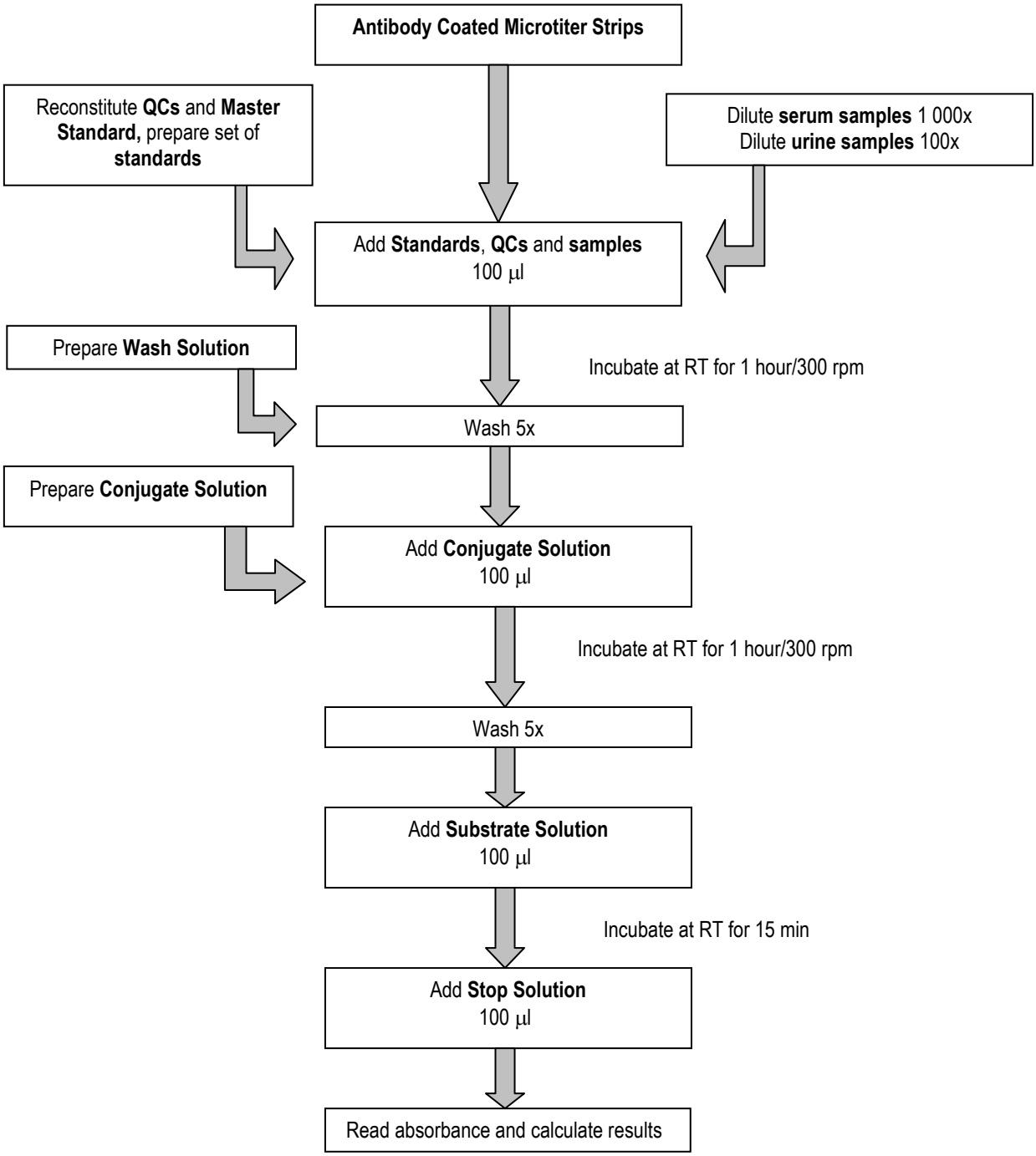
- Tvarijonaviciute A, Ceron JJ, Holden SL, Biourge V, Morris PJ, German AJ: Effect of weight loss in obese dogs on indicators of renal function or disease. *J Vet Intern Med*; **27**:31-38 (2013)

›› For more references on this product see our WebPages at www.biovendor.com

19. EXPLANATION OF SYMBOLS

REF	Catalogue number
Cont.	Content
LOT	Lot number
	Attention, see instructions for use
	Potential biological hazard
	Expiry date
	Storage conditions
	Name and registered office of the manufacturer

Assay Procedure Summary



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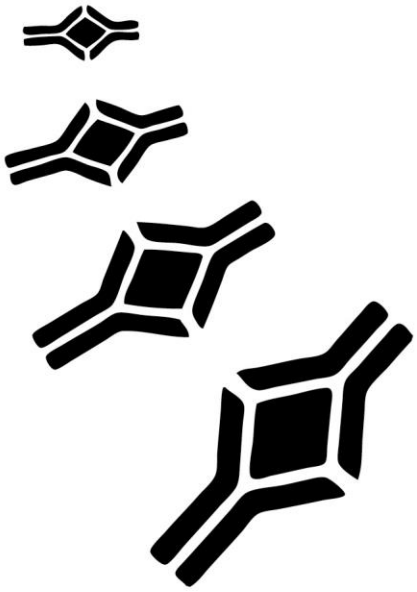

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