Canine Cystatin C ELISA

Cat. No.: BA2008

For research use only!

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

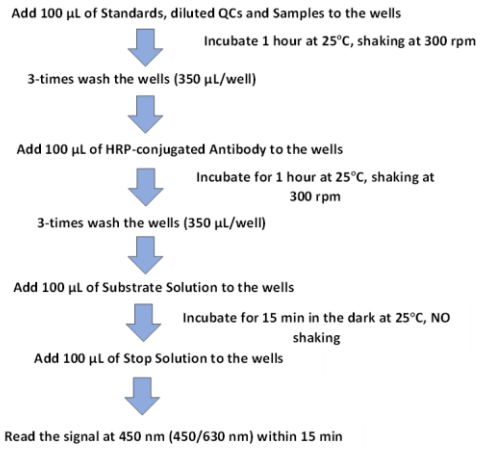
Cystatin C is a non-glycosylated basic protein belonging to the super-family of cysteine proteinase inhibitors. It consists of a single polypeptide chain having 120 amino acids.
It is produced by all nucleated cells within the body and is released during phagocytosis and inflammation. In the kidney, 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 clearance 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.

PRINCIPLE OF CANINE CYSTATIN C ELISA

The microtiter plate is coated with the antibody specifically binding the Canine Cystatin C. The Canine serum or urine is incubated in the plate with the capture antibody.

The specimen is washed out and the specifically bound protein is incubated with HRP-labelled detection antibody. Unbound reagent is then washed out. Horseradish peroxidase (HRP) bound in the complex reacts with the chromogenic substrate (TMB) creating the blue colour. The reaction is stopped by addition of STOP solution (H_2SO_4).

The absorbance values are measured at 450 nm (optionally 450/630 nm) and are proportional to the concentration of Canine Cystatin C in the specimen. The concentration of Cystatin C in unknown samples is determined from the calibration curve which is created by plotting the absorbance values against the standard concentration values.



Kit Contents

Item	***Qty.***
Antibody Coated Microtiter Plate	96 wells
Conjugate Diluent	13 mL
Ab-HRP Conjugate 50x conc.	0.26 mL
Master Standard (lyophilized)	2 vials
Quality Control A (lyophilized)	2 vials
Quality Control B (lyophilized)	2 vials
Dilution Buffer 10x conc.	13 mL
Wash Buffer 15x conc.	50 mL
Substrate Solution	13 mL
STOP Solution	13 mL

MATERIAL REQUIRED BUT NOT SUPPLIED

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

WARNINGS AND 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).

STORAGE CONDITIONS

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

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.

Preparation of Standards

Canine 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 Canine Cystatin C in the stock solution is 10 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 (lyophilised)	See CofA	10 ng/mL
Std2	300 µL of Std1	300 µL	5 ng/mL
Std3	300 µL of Std2	300 µL	2.5 ng/mL
Std4	300 µL of Std3	300 µL	1.25 ng/mL
Std5	300 µL of Std4	300 µL	0.625 ng/mL
Std6	300 µL of Std5	300 µL	0.31 ng/mL
Blank	-	200 µL	0 ng/mL

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.

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.

Ab-HRP Conjugate 1x

Prepare a working solution of Ab-HRP by adding 260 µL of Ab-HRP Conjugate 50x conc. to 13 mL of Conjugate Diluent. Mix well.

Preparation of samples

Canine serum or urine may be used with this assay. It is recommended to assay not-frozen samples. For long-term storage the serum and plasma samples should be frozen at minimum -70°C.

Recommended dilution of **serum** samples is 1:100. 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 (50x): 10 µL of Dilution A + 490 µL of Dilution Buffer, for both singlets and duplicates; Mix well, vortex is recommended.

Recommended dilution of **urine s**amples is 1:100. Add 5 µL of samples to 495 µL of Dilution Buffer.
Recommended dilution of urine samples from dog patients with serum creatinine > 1.4 mg/dl is minimum 1:1000. Two step dilution is recommended.
Dilution A (20x) for both singlets and duplicates: 5 µL of samples + 95 µL of Dilution Buffer.
Dilution B (50x): 10 µL of Dilution A + 490 µL of Dilution Buffer, for both singlets and duplicates; Mix well, vortex is recommended.

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

ASSAY PROCEDURE

1. Prepare the reagents as described in the previous chapter.
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3. Pipette 100 µL of set of Standards, Quality Controls, diluted Samples and Dilution Buffer = Blank into each well. Incubate for **1 hour** at 25°C ±2°C, shaking at 300 rpm.
4. 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.
5. Pipette 100 µL of HRP-labelled Antibody Conjugate into each well. Incubate for **1 hour** at 25°C ±2°C, shaking at 300 rpm.
6. Wash the wells as described in point 3.
7. Pipette 100 µL Substrate solution, incubate for **15 min,** at 25°C ±2°C**.** Avoid exposure to the light during this step.
8. Pipette 100 µL of STOP solution.
9. Read the signal at 450 or 450/630 nm within 15 min.

PERFORMANCE CHARACTERISTICS

Samples used in the tests were diluted 1:1000 as recommended and assayed. The results are multiplied by the dilution factor.

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 0,005 ng/mL of sample.

2. Precision

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

Sample	Mean (µg/mL)	SD (µg/mL)	CV (%)
1	0.68	0.06	8.4
2	4.60	0.19	4.2

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

Sample	Mean (µg/mL)	SD (µg/mL)	CV (%)
1	0.69	0.03	4.1
2	4.42	0.32	7.3

3. Accuracy

Dilution linearity

Sample	Dilution	Measured concentration (µg/mL)	Expected concentration (µg/mL)	Yield (%)
Serum 1		2.88	-	-
	2x	1.36	1.44	95
	4x	0.64	0.72	88
	8x	0.31	0.36	86
Serum 2		7.77	-	-
	2x	3.60	0.39	93
	4x	1.71	1.94	88
	8x	0.86	0.97	88
Urine 1		0.74	-	-
	2x	0.36	0.37	96
	4x	0.17	0.18	92
	8x	0.09	0.09	93
Urine 2		12.55	-	-
	2x	5.97	6.28	95
	4x	2.88	3.14	92
	8x	1.42	1.57	91

Spiking Recovery

Sample	Measured concentration (µg/mL)	Expected concentration (µg/mL)	Yield (%)
Serum 1	1.52	-	-
	2.10	2.02	104
	2.62	2.52	104
	3.63	3.52	103
Serum 2	1.05	-	-
	1.66	1.55	108
	2.11	2.05	103
	3.16	3.05	104
Urine 1	0.51	-	-
	0.60	0.57	106

	0.63	0.63	100
	0.79	0.76	104
Urine 2	0.16	-	-
	0.22	0.22	98
	0.30	0.28	107
	0.42	0.41	102

Definition of the standard

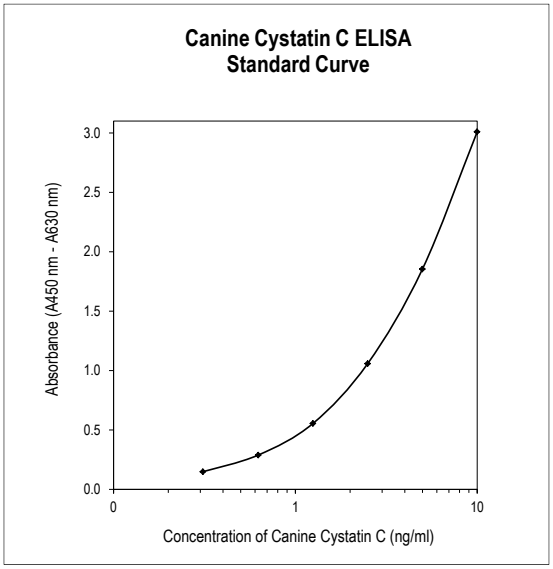
The recombinant canine Cystatin C is used as the Standard. The recombinant canine Cystatin C, produced in E.coli, is 14.85 kDa protein.

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 canine Cystatin C levels with the assay.

Typical standard curve

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.



RESOURCES

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