BioVendor Group ®

ENG

Instructions for Use:

CANINE PROCALCITONIN ELISA

Catalogue number:

RBL022R

For research use only!

BioVendor R&D®

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HISTORY OF CHANGES

Previous version	Current version
	ENG.001.A
New edition	

1.   INTENDED USE

Enzyme Immunoassay for the quantitative determination procalcitonin in canine 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

Procalcitonin (PCT), a polypeptide with a molecular mass of about 13 kDa, is the precursor of calcitonin. PCT is constitutively produced in the C cells of the thyroid gland and does not exhibit hormone activity [1].
PCT is markedly elevated within 2 to 4 hours in severe forms of systemic inflammation or in bacterial infections, and the level persists until recovery. The biological half-life of PCT is 22 to 26 hours, an advantageous time point compared with CRP and other acute-phase reactants [2]. Because up-regulation of PCT is attenuated by INF- γ, a cytokine released in response to viral infections, PCT is more specific for bacterial infections and may help to distinguish bacterial infections from viral illnesses [3].
It should be noted that PCT is also elevated in noninfectious conditions such as trauma, surgery, cardiogenic shock, burns, heat stroke, acute respiratory distress syndrome, infected necrosis after acute pancreatitis, and rejection after transplantation [4].
Severe bacterial infections can result in marked morbidity and death in veterinary patients, with 50–70% of dogs with sepsis succumbing to their disease. Early diagnosis of infection is essential for the appropriate management of sepsis, as it allows rapid administration of antibiotics resulting in improved outcomes [5]. Although PCT mRNA expression from nonthyroidal tissue has been demonstrated in dogs with inflammation, sepsis and SIRS [6, 7], very little is known about serum PCT concentration in dogs due to the lack of a validated canine assay [5].

4.   TEST PRINCIPLE

In the Canine Procalcitonin ELISA kit, standards and samples are incubated in microtitration wells pre-coated with polyclonal anti-canine procalcitonin antibody. After 60 minutes incubation followed by washing, biotin-labelled polyclonal anti-canine procalcitonin antibody is added and incubated with the captured procalcitonin 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 canine procalcitonin. A standard curve is constructed by plotting absorbance values against procalcitonin concentrations of standards and concentrations of unknown samples are determined using this standard curve.

5. PRECAUTIONS

− For research use only
− For professional laboratory use
− The reagents with different lot numbers should not be mixed
− To prevent cross sample contamination, use disposable labware and pipette tips
− To protect laboratory stuff, wear protective gloves and protective clothing
− The substrate solution should remain colourless, keep it protected from light
− 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 (lyophilized)	1 vial
Biotin-Ab Diluent	13 mL
Master Standard (lyophilized)	2 vials
Dilution Buffer	2x10 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

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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

8.1.1   Canine Procalcitonin 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 procalcitonin in the stock solution is 800 pg/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 800 ng/mL (lyophilised)	See CoA	800 pg/mL
Std2	300 µL of Std1	300 µL	400 pg/mL
Std3	300 µL of Std2	300 µL	200 pg/mL
Std4	300 µL of Std3	300 µL	100 pg/mL
Std5	300 µL of Std4	300 µL	50 pg/mL
Std6	300 µL of Std5	300 µL	25 pg/mL
Blank	-	200 µL	0 pg/mL

8.2   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$_2$O). Mix well. Store at 4°C for two weeks or at -20°C for long term storage.

8.3   Biotin Labelled Antibody 1x

Refer to the Certificate of Analysis for current volume of Biotin-Ab Diluent needed for reconstitution of Biotin Labelled Antibody!!! Reconstitute the lyophilized Biotin Labelled Antibody with Biotin-Ab Diluent just prior to the assay. Let it dissolve at least 15 minutes with occasional gentle shaking (not to foam).
Dilute Biotin Labelled Antibody Concentrate (100x) with Biotin-Ab Diluent (e.g. 130 µL of Biotin Labelled Antibody Concentrate + 13 mL of Biotin-Ab Diluent for 96 wells).

9.   PREPARATION OF SAMPLES

Canine serum, plasma 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 -70oC and the urine samples should be frozen in protective medium at minimum -70°C. Recommended dilution of samples is 1:5, i.e., for singlets 30 µL of sample + 120 µL of Dilution Buffer, for duplicates 60 µL of samples + 240 µL of Dilution Buffer, respectively.

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 25°C ±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 **15 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 1:5 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 procalcitonin giving absorbance higher than absorbance of blank + 3 standard deviations, is better than 4 pg/mL of sample.

11.2 Specificity

Less than 10% cross-reactivity was observed with recombinant porcine procalcitonin at concentration 1 ng/mL. Less than 1% cross-reactivity was observed with recombinant monkey (Macaca mulatta) procalcitonin at concentration 1 ng/mL. No significant cross-reactivity was observed for tested serum samples of other mammalian species.

11.3 Precision

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

Sample	Mean (pg/mL)	SD	CV (%)
1	528	19.7	3.7
2	242	11.0	4.6

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

Sample	Mean (pg/mL)	SD	CV (%)
1	512	34.2	6.7
2	222	16.6	7.5

11.4 Accuracy

11.4.1 Dilution linearity

Sample	Dilution	Measured concentration (pg/mL)	Expected concentration (pg/mL)	Yield (%)
Serum 1		377	-	-
	2x	185	189	98
	4x	93	94	99
	8x	47	47	100
Serum 2		480	-	-
	2x	246	240	103
	4x	120	120	100
	8x	61	60	101
Urine 1		504	-	-
	2x	232	252	92
	4x	116	126	92
	8x	62	63	98

11.4.2 Spiking Recovery

Sample	Spike (ng/mL)	Measured concentration (pg/mL)	Expected concentration (pg/mL)	Yield (%)
Serum 1	-	218	-	-
	0.5	304	342	89
	1.0	443	468	95
	2.0	684	717	95
Urine 1	-	128	-	-
	0.5	241	253	95
	1.0	354	378	94
	2.0	599	628	95

11.4.3 Definition of the standard

In this assay the recombinant protein (E. coli) is used as the standard. The recombinant procalcitonin is a 12.7 kDa protein consisting of 105 amino acid residues of the canine procalcitonin (UniProtKB acc.no. P41547 (Ala26-Arg130) and 10 extra aminoacids.

11.4.4 Reference range

Mean concentration of canine procalcitonin in serum samples from 24 healthy donors (beagle dogs) was 281 +/- 115 pg/mL (median 260 pg/mL). However, 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 reference ranges for canine procalcitonin levels with the assay.

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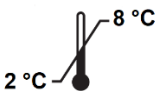
12. CALCULATION

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.

Canine Procalcitonin Standard Curve
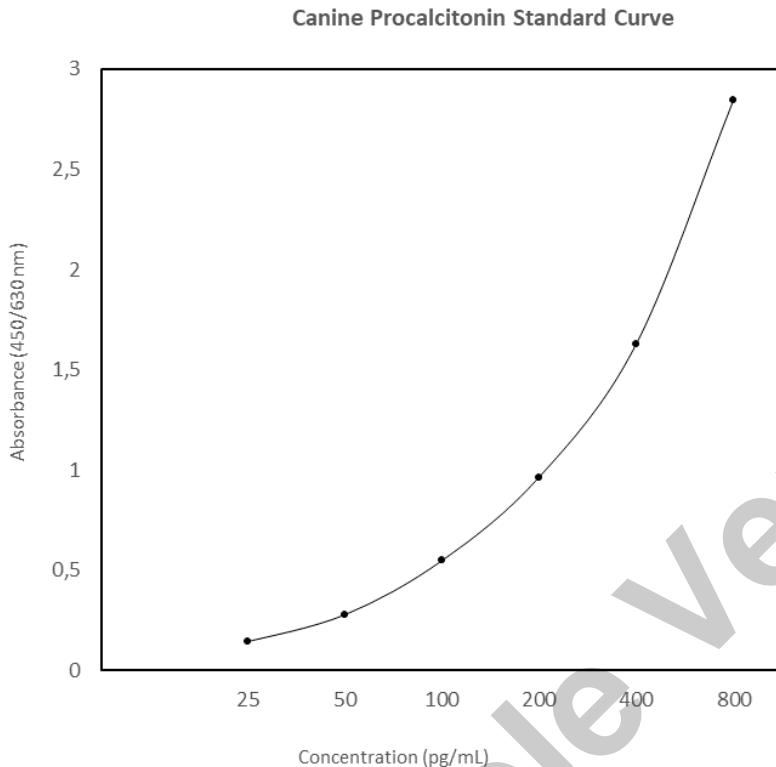
13. REFERENCES

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14. EXPLANATION OF SYMBOLS

REF	Catalogue number
LOT	Batch code
⚠	Caution
⧗	Use by date
2 °C — 8 °C	Temperature limit
🏭	Manufacturer
📖 www.biovendor.com	Read electronic instructions for use - eIFU
Σ 96	The content is sufficient for 96 tests
☣	Biological risks

15. ASSAY PROCEDURE - SUMMARY

Add 100 µL of Standards, diluted QCs and Samples to the wells

Incubate 1 hour at 25°C, shaking 300 rpm

3-times wash the wells (350 µL/well)

Add 100 µL of Biotin-labelled Antibody to the wells

Incubate for 1 hour at 25°C, shaking at 300 rpm

3-times wash the wells (350 µL/well)

Add 100 µL of SAV-HRP to the wells

Incubate for 30 min at 25°C, shaking at 300 rpm

3-times wash the wells (350 µL/well)

Add 100 µL of Substrate Solution to the wells

Incubate for 15 min in the dark at 25°C, NO shaking

Add 100 µL of Stop Solution to the wells

Read the signal at 450 nm (450/630 nm) within 15 min

	A	B	C	D	E	F	G	H
12								
11								
10								
9								
8								
7								
6								
5								
4								
3								
2								
1								

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