Dog Procalcitonin ELISA PCT-4

INTRODUCTION

Procalcitonin (PCT) is a serum biomarker of bacterial infections and sepsis. In studies at Life Diagnostics, we found levels of 0.5 to 2 ng/ml in healthy dogs. In dogs with infections, we found levels up to 150 ng/ml.

PRINCIPLE OF THE ASSAY

The assay uses two dog Procalcitonin antibodies developed at Life Diagnostics. One is used as coating antibody. The other is conjugated to HRP and used for detection. The standard is prepared from recombinant dog Procalcitonin. Standards and diluted samples (100 µl) are incubated in anti-PCT coated microtiter wells for 45 minutes. After washing the wells, HRP-conjugate (100 µl) is added and incubated for 45 minutes. If PCT molecules are present, they are sandwiched between the capture and detection antibodies. The wells are then washed to remove unbound HRP-conjugate. TMB is added and incubated for 20 minutes. If PCT is present, a blue color develops. Color development is stopped after 20-minutes by addition of Stop Solution, changing the color to yellow. Absorbance is measured at 450 nm. The concentration of PCT is proportional to absorbance and is derived from a standard curve.

MATERIALS

Materials provided with the kit:

- Anti-PCT coated plate (12 x 8-well strips)
- Anti-PCT HRP conjugate, 11 ml
- PCT stock, 1 vial. **Store at -80°C**
- 20x Wash Solution: TBS50-20, 50 ml
- Diluent: YD50-1, 2 x 50 ml
- TMB: TMB11-1, 11 ml
- Stop Solution: SS11-1, 11 ml

Materials required but not provided:

- Pipettors and tips
- Distilled or deionized water
- Polypropylene tubes or 96-well polystyrene plates
- Vortex mixer
- Absorbent paper or paper towels
- Plate incubator/shaker
- Plate washer
- Plate reader capable of measuring absorbance at 450 nm
- Graphing software

STORAGE

Immediately store the PCT stock in a -80°C freezer on receipt. The remainder of the kit should be stored at 4°C and the microtiter plate should be kept in a sealed bag with desiccant. The kit will remain stable for six months from the date of purchase.

GENERAL INSTRUCTIONS

1. All reagents should be allowed to reach room temperature before use.
2. It is important that standards and samples be added to the ELISA plate quickly. If testing large numbers of samples, rather than pipetting standards and samples from individual tubes into the ELISA plate, we recommend the following: pipette an excess volume of standards and samples into wells of a blank polystyrene 96-well plate[1]. Then use an 8 or 12-channel multi-pipettor to quickly transfer 100 µl aliquots to the wells of the antibody-coated plate.
3. The wash procedure is critical. Insufficient washing will result in poor precision and falsely elevated absorbance readings.
4. Laboratory temperature will influence absorbance readings. The assay was calibrated using a shaking incubator set at 150 rpm and 25°C. Performing the assay at lower temperatures and mixing speeds may result in lower absorbance values.

WASH SOLUTION

The Wash Solution is provided as a 20x stock. Prior to use, dilute the contents of the bottle (50 ml) with 950 ml of distilled or deionized water. Unused Wash Solution may be stored at 4°C for one week.

DILUENT

The Diluent (YD50-1) is formulated for measurement of PCT in serum. It is supplied ready to use. DO NOT substitute other reagents.

STANDARD

1. The stock is provided as a frozen liquid. Thaw the stock at room temperature a few minutes before preparing the working standards. Return the stock to a -80°C freezer immediately after use. It is comprised of pure PCT in a stabilizing matrix.
2. Prepare the 25 ng/ml standard as described on the stock vial label.
3. Label seven polypropylene tubes as 12.5, 6.25, 3.13, 1.56, 0.78, 0.39 and 0 ng/ml. Dispense 0.25 ml of diluent into each.
4. Pipette 0.25 ml of the 25 ng/ml PCT standard into the tube labeled 12.5 ng/ml and mix. This provides the 12.5 ng/ml PCT standard.
5. Similarly prepare the 6.25 to 0.39 ng/ml standards by two-fold serial dilution.

SAMPLES

The assay is intended for measurement of PCT in dog serum. In serum from sick dogs, we found PCT levels up to 150 ng/ml. We recommend testing serum at a dilution of 10-fold, but optimal dilutions should be determined by the end user. To avoid matrix effects, do not use dilutions lower than 10-fold. Diluent YD50-1 must be used for sample dilution.

[1] Standards and sample dilutions may also be prepared directly in a blank polystyrene plate.

HRP CONJUGATE

The HRP conjugate is provided as a concentrated stock. Dilution instructions are listed on the vial label. Diluent YD50-1 must be used for dilution. Use 100 µl per well.

PROCEDURE

1. Secure the desired number of 8-well strips in the cassette. Unused strips should be stored in a sealed bag with desiccant at 4°C.
2. Dispense 100 µl of standards and diluted samples into appropriate wells. We recommend that standards and samples be tested in duplicate.
3. Incubate on a plate shaker at 150 rpm and 25°C for 45 minutes.
4. Empty and wash the microtiter wells 5 times with 1x Wash Solution using a plate washer (400 µl/well).
5. Dispense 100 µl of diluted HRP conjugate into the wells.
6. Incubate on a plate shaker at 150 rpm and 25°C for 45 minutes.
7. Empty and wash the microtiter wells 5 times with 1x Wash Solution using a plate washer (400 µl/well).
8. If necessary, strike the wells sharply onto absorbent paper or paper towels to remove all residual droplets.
9. Dispense 100 µl of TMB into each well.
10. Incubate on an orbital micro-plate shaker at 150 rpm at 25°C for 20 minutes.
11. After 20 minutes stop the reaction by adding 100 µl of Stop Solution to each well.
12. Gently mix. It is important to make sure that all the blue color changes to yellow.
13. Read absorbance at 450 nm[2] with a plate reader within 5 minutes.

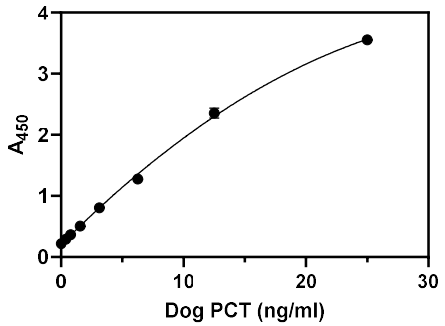
RESULTS

1. Using curve fitting software, construct a standard curve by plotting absorbance values of the standards versus the PCT concentration. We suggest fitting data to a second order polynomial equation.
2. Derive the concentration of PCT in the samples and multiply by the dilution factor to determine the concentration in the original sample.
3. If the absorbance values of samples fall outside the standard curve, samples should be further diluted appropriately and re-tested.

TYPICAL STANDARD CURVE

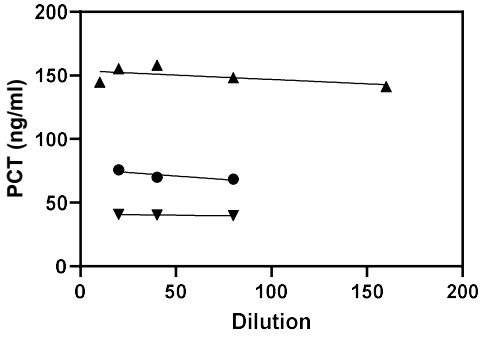
A typical standard curve is shown below. This curve is for illustration only.

PCT (ng/ml)	A_{450}
25	3.554
12.5	2.357
6.25	1.277
3.13	0.807
1.56	0.504
0.78	0.364
0.39	0.291
0	0.219



PERFORMANCE

Linearity: To assess the linearity of the assay, serum samples with PCT concentrations of 38, 71 and 147 ng/ml diluted with YD50-1 to give values within range of the assay.



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For technical assistance please email us: techsupport@lifediagnostics.com

[2] If absorbance of the high standard is ≥4 when measured at 450 nm, absorbance of all standards and samples should be read at 405 nm.

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