# DOG PROCALCITONIN (PCT) SPARCL™ ASSAY
# Life Diagnostics, Inc., Catalog Number: PCT-SP-4

## INTRODUCTION

Procalcitonin (PCT), a protein of ~14 kDa, is a precursor of the calcium regulating hormone calcitonin. Serum levels of PCT increase during bacterial infection. In humans it is used as a biomarker of sepsis. PCT mRNA levels increase in dogs with lymphoma, infection, and inflammation (ref 1). However, prior to the launch of this kit, the availability of a suitable immunoassay has frustrated efforts to measure PCT protein levels in dogs (ref 2).

## PRINCIPLE OF THE ASSAY

The dog PCT SPARCL™[1] (Spatial Proximity Analyte Reagent Capture Luminescence, ref 3) assay uses two dog PCT specific antibodies. One is conjugated to horseradish peroxidase (HRP), the other is conjugated to acridan, a chemiluminescent substrate. When the HRP and acridan conjugated antibodies bind to PCT they are brought into close proximity. With the addition of hydrogen peroxide (trigger solution), HRP catalyzes oxidation of proximal acridan molecules causing a flash of chemiluminescence. Acridan conjugated antibodies distant from HRP produce no signal. This principle allows the development of a homogeneous assay that allows rapid measurement of PCT concentrations.

Diluted serum samples and standards are mixed with the HRP and acridan-conjugated antibodies in the wells of the 96-well SPARCL™ plate[2] provided with the kit. After incubation for 30 minutes on a shaker at 25°C and 150 rpm, the plate is placed into a luminometer. Trigger solution containing hydrogen peroxide is injected into each well and luminescence is immediately measured. The concentration of PCT is proportional to luminescence and is derived from a standard curve.

## MATERIALS AND COMPONENTS

*Materials provided with the kit:*

- Anti-PCT HRP conjugate. **Store ≤ -70°C**
- Anti-PCT acridan conjugate. **Store ≤ -70°C**
- Dog PCT stock. **Store ≤ -70°C**
- Diluent; CSD50-1, 2 x 50 ml
- Trigger solution; TS7-1, 7 ml
- White SPARCL™ plate (12 x 8-well)
- Clear untreated 96-well plate

*Materials required but not provided:*

- Precision pipettes and tips
- Polypropylene tubes
- Vortex mixer
- Microplate incubator/shaker
- Luminometer capable of simultaneous injection & measurement
- Graphing software

## STORAGE

Store the HRP conjugate, acridan conjugate and PCT stock at -70°C (they may be stored at -20°C for one week). The remainder of the kit should be stored at 2-8°C. The SPARCL™ plate should be kept in a sealed bag with desiccant and antioxidant. The kit will remain stable for at least six months from the date of purchase, provided that the components are stored as described.

## GENERAL INSTRUCTIONS

1. Please take the time to completely read all instructions before starting your assay. Contact us if you need clarification.
2. All reagents used in the assay should be allowed to reach room temperature (25°C) before use.
3. It is important that standards and samples be added to the SPARCL™ plate quickly. If testing large numbers of samples, rather than pipetting standards and samples directly into the white SPARCL™ plate using a single channel pipettor, we recommend the following. First, pipette an excess volume of standards and samples into appropriate wells of the clear 96-well plate. Then use an 8- or 12-channel multipipettor to quickly and efficiently transfer 50 µl aliquots to the appropriate wells of the white SPARCL™ plate. The wells of the clear plate hold a maximum volume of 300 µl.
4. Follow the sequence of events below when running the assay.

Prime and program the Luminometer
⇩
Prepare standards and diluted samples
⇩
Prepare HRP + Acridan conjugate mix
⇩
Add HRP + Acridan conjugate mix to the wells (25 µl)
⇩
Add standards and samples to the wells (50 µl)
⇩
Incubate plate at 150 rpm/25°C for 30 min
⇩
Measure luminescence after injection of Trigger (37.5 µl)

## STANDARD PREPARATION

The dog PCT stock is comprised of purified PCT in a stabilizing carrier protein matrix. Thaw the stock shortly before use.

1. Label 8 polypropylene tubes as 25, 12.5, 6.25, 3.125, 1.563, 0.781, 0.390 and 0 ng/ml.
2. Into the tube labeled 25 ng/ml, pipette the volume of diluent detailed on the stock vial label. Then add the indicated volume of stock and mix gently. This provides the 25 ng/ml standard.
3. Dispense 150 µl of diluent into the tubes labeled 12.5, 6.25, 3.125, 1.563, 0.781, 0.390 and 0 ng/ml.
4. Pipette 150 µl 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 remaining standards by serial dilution.

**Please Note: Use the standards within one hour of preparation.**

## SAMPLE PREPARATION

Serum samples should be diluted at least 10-fold with diluent in order to avoid martrix effects. Plasma should not be used. Serum from dogs without bacterial infection had undetectable levels of PCT. However,

[1] The SPARCL technology was developed by Lumigen Corp.

[2] The plate provided with the kit has been treated with a reagent that reduces background chemiluminescence. Untreated plates cannot be used.

in a panel of serum from sick dogs we found samples with PCT levels ranging from 25 – 2000 ng/ml. Optimal dilutions must be determined by the end user. Use only the diluent provided with the kit.

## CONJUGATE MIX PREPARATION

Instructions for preparation of the conjugate mix are detailed on the box that contains the HRP and acridan conjugates. If necessary, after thawing, briefly centrifuge to ensure that the contents are at the bottom of the tubes. Prepare the mix shortly before use using the diluent provided with the kit.

## LUMINOMETER SETUP

1. The luminometer must be capable of injection and simultaneous measurement of luminescence without any delay.
2. Prime the luminometer injection port with 1 ml of trigger solution.
3. Place the injection needle into the injection port as needed for BMG luminometers.
4. Program the luminometer to inject 37.5 µl of trigger solution per well and to measure from time zero for 1 second (50 x 0.02 second intervals).
5. Define the format of the assay using the luminometer software.
6. Because the white SPARCL™ plate is provided as a 12 x 8-well strips, allowing use of fewer than 96-wells, make sure that the luminometer is programmed to inject trigger solution only into the wells being used.
7. We use a BMG LUMIstar Omega set at a gain of 3600. Optimal gain should be determined by the end user.
8. There are a number of manufacturers of luminometers that are equipped to run a SPARCL™ assay. Please contact Life Diagnostics or Lumigen (www.lumigen.com) to discuss your luminometer.

## PROCEDURE

1. Before starting the assay ensure that the luminometer is primed with trigger solution and that the injection needle is positioned in the injection port.
2. Secure the desired number of SPARCL™ 8-well strips in the holder. Immediately seal unused strips in the resealable bag with desiccant and antioxidant. Store unused strips at 2-8°C.
3. Aliquot 25.0 µl of conjugate mix into each well.
4. Dispense 50.0 µl of standards and diluted samples into the wells (we recommend that standards and samples be tested in duplicate).
5. Incubate on an orbital micro-plate shaker at 150 rpm 25°C for 30 minutes.
6. After the 30-minute incubation, place the plate in the luminometer and measure luminescence after injection of trigger solution (37.5 µl).
7. Remove the plate from the luminometer and discard the used strips. Keep the plate frame if future use is intended.

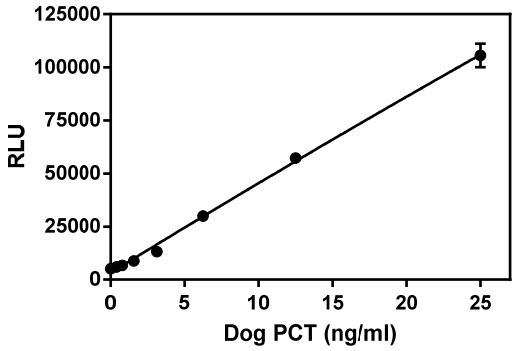
## CALCULATION OF RESULTS

1. Before calculating results, review the raw data. If artefacts (RLU spikes) are apparent immediately after injection of trigger solution, eliminate that portion of the luminescence profile from analysis for all wells. We routinely use the sum of RLU values from a 100-980 ms data collection window.
2. Determine the sum of RLU values within the data collection window for the standards and samples.
3. Using graphing software, construct a standard curve by plotting the sum of RLU values (RLU) for the standards versus the PCT concentration in ng/ml.
4. Fit data using graphing software. We find that either a second order polynomial model or a single site, total and nonspecific binding model work well.
5. Derive the corresponding concentration of PCT in the diluted samples from the standard curve.
6. Multiply the derived concentration by the dilution factor to determine the concentration of PCT in the serum or plasma sample.
7. If the RLU values of diluted samples fall outside the standard curve, the original samples should be diluted appropriately and re-tested.

## TYPICAL STANDARD CURVE

A typical standard curve with RLU plotted on the Y-axis versus PCT concentrations on the X-axis is shown below. This curve is for illustration only and should not be used to calculate unknowns.

| PCT(ng/ml) | RLU |
|---|---|
| 25 | 105573 |
| 12.5 | 57201 |
| 6.25 | 29994 |
| 3.125 | 13191 |
| 1.563 | 8762 |
| 0.781 | 6679 |
| 0.390 | 6004 |
| 0 | 5122 |




## REFERENCES

1. Kuzi S. et al. Canine procalcitonin messenger RNA expression. J Vet Diagn Invest. 20:629-633 (2008)
2. Floras ANK. et al. Investigation of a commercial ELISA for the detection of canine procalcitonin. J Vet Intern Med. 28:599-602 (2014)
3. Akhavan-Tafti H. et al. A homogeneous chemiluminescent immunoassay method. J Am Chem Soc. 20;135(11):4191-4 (2013)

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