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, 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™ plate2 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-dog PCT HRP conjugate.
- Anti-dog PCT acridan conjugate.
- Dog PCT stock.
- Diluent (CSD50-1), 2 x 50 ml
- Trigger solution, 7 ml
- White SPARCL™ plate (12 x 8-well)
- Clear untreated 96-well plate

Materials required but not provided:
- Reagent alcohol3
- Precision pipettes and tips
- Polypropylene tubes
- Vortex mixer
- Micro-Plate incubator/shaker
- Luminometer capable of simultaneous injection & measurement
- PC graphing software

STORAGE

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.
3 Reagent Alcohol consists of ethanol, isopropyl alcohol and methanol in the ratio 90:5:5.
4 Dilutions of standards can be performed in wells A1-A8 of the clear untreated 96-well plate provided with the kit. This allows rapid transfer of standards to the white SPARCL™ plate using a multipipettor. Diluted samples can also be first aliquoted into appropriate wells of the clear polystyrene plate and subsequently transferred to the SPARCL™ plate with a multipipettor. If using this method, ensure that an excess volume is aliquoted into the clear plate in order to ensure complete transfer of 50 μl aliquots to the SPARCL™ plate.

SPARCL™ plate using a multipipettor. Diluted samples can also be first aliquoted into appropriate wells of the clear polystyrene plate and subsequently transferred to the SPARCL™ plate with a multipipettor. If using this method, ensure that an excess volume is aliquoted into the clear plate in order to ensure complete transfer of 50 μl aliquots to the SPARCL™ plate.

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 matrix effects. Plasma should not be used. Serum from dogs without bacterial infection had undetectable levels of PCT. However, 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 LUMItstar 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.
2. Using graphing software, construct a standard curve by plotting the luminescence (RLU) for the standards versus the PCT concentration in ng/ml.
3. Fit data using graphing software. We find that second order polynomial model works well.
4. Derive the corresponding concentration of PCT in the diluted samples from the standard curve.
5. Multiply the derived concentration by the dilution factor to determine the actual concentration of PCT in the serum or plasma sample.
6. If the RLU values of diluted samples fall outside the standard curve, 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 the purpose of illustration only and should not be used to calculate unknowns.

- **REFERENCES**
  

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

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