

# Dog CRP Sirius SPARCL Kit, Cat. No. CRP-SPL-4

## INTRODUCTION

CRP (C-reactive protein) levels increase in dog serum because of injury, infection and cancer. CRP can therefore be used to diagnose and monitor disease. The Sirius SPARCL™<sup>1</sup> assay allows simple and rapid measurement of dog CRP using a Berthold Sirius-L single tube luminometer.

## PRINCIPLE OF THE ASSAY

The assay uses two CRP-specific antibodies. One conjugated to horseradish peroxidase (HRP), the other to acridan, a chemiluminescent substrate. When HRP and acridan conjugated antibodies bind to CRP they are brought into proximity. Upon addition of hydrogen peroxide, HRP catalyzes oxidation of proximal acridan molecules causing a flash of luminescence that is proportional to CRP concentration.

Diluted serum samples (100 µl) are dispensed into test tubes and mixed with 0.5 ml of combined acridan and HRP conjugates. After 45 minutes, tubes are placed in the Sirius-L luminometer. Luminescence is measured after injection of background reducer, that eliminates nonspecific luminescence, followed by trigger-solution containing hydrogen peroxide. The concentration of CRP is determined from the ratio of sample luminescence to the that of a dog CRP standard.

## MATERIALS AND COMPONENTS

### Materials provided with the kit:

- Acridan & HRP conjugates, 1 vial **Store at -20°C**
- Dog CRP standard, 1 vial **Store at -20°C**
- Diluent; CSD50-1, 50 ml
- Background reducer; BR4-1, 4 ml
- Trigger solution; TS4-1, 4 ml
- 15 ml centrifuge tubes, 4

### Materials required but not provided:

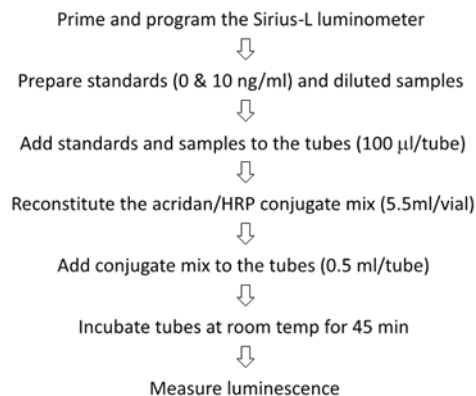
- Sirius-L luminometer with two injectors and PC
- Precision pipettors and tips
- Test tubes (borosilicate, 12 x 75 mm)
- 12 x 75 mm test tube racks
- Excel software

## STORAGE

Store the conjugate and standard vials at or below -20°C. The remainder of the kit should be stored at 2-8°C.

## 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 removed from the refrigerator or freezer and allowed to reach room temperature (25°C) before use.
3. Follow the sequence of events below when running the assay.



## LUMINOMETER SETUP

1. Turn the Sirius-L luminometer on and allow it to warm up for 15 minutes.
2. Place the tubing from injector one into a 15 ml centrifuge tube containing background reducer.
3. Place the tube from injector two into a 15 ml centrifuge tube containing trigger solution.
4. Open the Sirius-L software on the computer. The Protocol Manager screen will load.
5. Click on "Priming Injectors" and program the luminometer to prime injector-1 with 3 x 0.5 ml of background reducer and injector-2 with 3 x 0.5 ml of trigger solution. Make sure to select "to tube" as the destination.
6. Open the luminometer drawer and insert an empty 12 x 75 mm test tube.
7. Close the drawer and click "Prime"
8. Wait for priming to complete then open the drawer and discard the tube.
9. Highlight the Quick Measurement program in the Protocol Manager window and click "Edit". Enter the following parameters:

Delay time:	Measurement time:	Save			
2.6s	0.8s				
Injectors					
#1	Volume [ul]	Delay [s]	#2	Volume [ul]	Delay [s]
<input checked="" type="checkbox"/>	50	1	<input checked="" type="checkbox"/>	100	2

10. Click "Save".
11. Highlight Quick Measurement and click "Run".
12. The luminometer is now ready for use.

## ASSAY FORMAT

1. Download the Excel spreadsheet from the Life Diagnostics website: <https://lifediagnostics.com/dog-crp-spreadsheet/>
2. The first two rows/tubes are reserved for the zero and 10 ng/ml standards.

<sup>1</sup> SPARCL technology, using acridan- and HRP-conjugated antibodies, was developed by and is licensed from Lumigen Corp.

- Enter your sample identities and dilution factor(s) (we recommend 500-fold) into rows 3+ in the order that you intend to measure them.

### SAMPLE PREPARATION

We recommend that serum or plasma be tested initially at a dilution of 500-fold:

- For each sample dispense 998  $\mu$ l of CSD50-1 diluent into a 12 x 75 mm glass test tube.
- Mix 2.0  $\mu$ l of serum or plasma with 998  $\mu$ l of diluent to give a 500-fold dilution.

### STANDARD PREPARATION

- Reconstitute the 10 ng/ml CRP standard with the volume of CSD50-1 diluent indicated on the vial label.
- 100  $\mu$ l is pipetted into tube 2 as described in the Procedure section.

### CONJUGATE MIX PREPARATION

- The acridan and HRP conjugate mix should be prepared just before use (step 6 in the Procedure section).
- Add 5.5 ml of diluent CSD50-1 to the vial that contains the freeze-dried conjugates and mix gently by inversion 20x.
- Each vial of reconstituted conjugate mix provides sufficient reagent to measure 0 and 10 ng/ml standards and up to eight samples.<sup>2</sup>

### PROCEDURE

- Before starting the assay ensure that the luminometer is primed with background reducer and trigger solution and that the SPARCL program is loaded (refer to the Luminometer Setup section).
- Prior to starting the assay ensure that the 12 x 75 mm borosilicate test tubes fit easily in the luminometer cuvette holder.
- Pipet 100  $\mu$ l of diluent into tube one. This serves as the zero standard.
- Pipet 100  $\mu$ l of the reconstituted 10 ng/ml CRP standard into tube two.
- Pipet 100  $\mu$ l aliquots of the diluted samples into tubes 3, 4, 5... as defined by your assay format.
- Add 0.50 ml of freshly prepared conjugate mix to each tube and mix gently. A vortex mixer may be used if available.
- Incubate the mixtures at room temperature.
- After 45 minutes insert tube 1 into the sample holder of the luminometer and close the drawer. The luminometer automatically injects background reducer and trigger solution, then measures luminescence (RLU/s).
- Once the RLU/s value is recorded on the screen open the drawer and discard the tube.
- Determine luminescence for the remaining tubes.

### CALCULATION OF RESULTS

- Cut and paste the RLU/s values into the RLU/s column of the downloaded Excel spreadsheet.
- CRP values ( $\mu$ g/ml) are automatically calculated.

### TYPICAL RESULTS

Typical results for the 0 and 10 ng/ml standards and twelve dog serum samples are shown below. Note that samples 2, 6, 7 and 12 gave RLU/s values significantly greater than the 10 ng/ml standard. It is likely that CRP concentrations are underestimated for these samples. Re-testing at a dilution of 10,000-fold would be recommended.

Dog CRP SIRIUS Worksheet						
Tube	Sample ID	Comments	Dilution	RLU/s	Zero subtracted RLU/s	$\mu$ g/ml
1	0 ng/ml CRP			10550	0	
2	10 ng/ml CRP			286122	275572	
3	1		500	125488	114938	2.09
4	2		500	552984	542434	9.84
5	3		500	43180	32630	0.59
6	4		500	253684	243134	4.41
7	5		500	252950	242400	4.40
8	6		500	1810418	1799868	32.66
9	7		500	556146	545596	9.90
10	8		500	44774	34224	0.62
11	9		500	208638	198088	3.59
12	10		500	83588	73038	1.33
13	11		500	246202	235652	4.28
14	12		500	671568	661018	11.99

### LUMINOMETER MAINTENANCE

The luminometer injectors should be cleaned with distilled or deionized water at the end of each day of use.

- Exit the Quick Measurement screen and click on "Priming Injectors". Enter the following parameters:

- Click "Prime". The contents of the injectors will be returned to the respective tubes. If future use is intended store the sealed tubes in a refrigerator.
- Place the tubing from injectors one and two into separate tubes containing distilled or deionized water.
- Enter the following parameters:

- Insert an empty 12 x 75 mm test tube into the luminometer. Close the drawer and click "Prime".
- After priming is complete discard the 12 x 75 mm tube and its contents.

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<sup>2</sup> If using multiple vials of conjugates to measure more than eight samples, combine the reconstituted contents of all vials and mix briefly before dispensing 0.5 ml

aliquots into the reaction tubes. Larger volumes of background reducer and trigger solution must also be used.