Rabbit CRP Sirius SPARCL Kit, Cat. No. CRP-SPL-10

INTRODUCTION

CRP (C-reactive protein) levels increase in rabbit serum because of injury and infection. In rabbits, levels can increase several hundred-fold. It has been used to monitor health of rabbits (ref 1) and as a biomarker in vaccine toxicity studies (ref 2). The Sirius SPARCL™[1] assay allows simple and rapid measurement of rabbit CRP using a Berthold Sirius-L single tube luminometer.

PRINCIPLE OF THE ASSAY

The assay uses two affinity purified rabbit CRP antibodies. One is 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 rabbit CRP standard.

MATERIALS AND COMPONENTS

Materials provided with the kit:

- Acridan & HRP conjugates, 1 vial Store at -20°C
- Rabbit 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.

Prime and program the Sirius-L luminometer

⇩

Prepare standards (0 & 10 ng/ml) and diluted samples

⇩

Add standards and samples to the tubes (100 µl/tube)

⇩

Reconstitute the acridan/HRP conjugate mix (5.5ml/vial)

⇩

Add conjugate mix to the tubes (0.5 ml/tube)

⇩

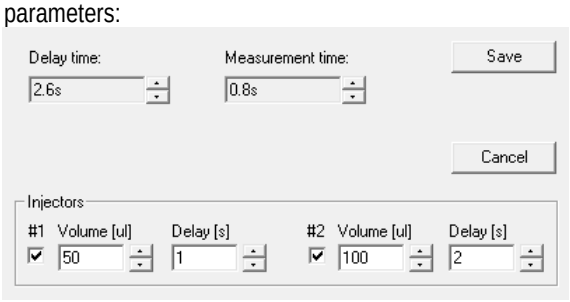
Incubate tubes at room temp for 45 min

⇩

Measure luminescence

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:



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/rabbit-crp-spreadsheet/
2. The first two rows/tubes are reserved for the zero and 10 ng/ml standards.

[1] SPARCL technology, using acridan- and HRP-conjugated antibodies, was developed by and is licensed from Lumigen Corp.

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

SAMPLE PREPARATION

Because serum levels of CRP can range from 0.2 to >100 μg/ml, we recommend that samples be tested at a minimum of two dilutions. We recommend dilutions of 200 and 16,000-fold.

1. For each sample dispense 995 μl and 395 μl of CSD50-1 diluent into separate tubes.
2. Mix 5.0 μl of serum with 995 μl of diluent to give a 200-fold dilution.
3. Mix 5.0 μl of the 200-fold diluted sample with 395 μl of diluent to give a 16,000-fold dilution.

STANDARD PREPARATION

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

CONJUGATE MIX PREPARATION

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

PROCEDURE

1. 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).
2. Prior to starting the assay ensure that the 12 x 75 mm borosilicate test tubes fit easily in the luminometer cuvette holder.
3. Pipet 100 μl of diluent into tube one. This serves as the zero standard.
4. Pipet 100 μl of the reconstituted 10 ng/ml CRP standard into tube two.
5. Pipet 100 μl aliquots of the diluted samples into tubes 3, 4, 5… as defined by your assay format.
6. Add 0.50 ml of freshly prepared conjugate mix to each tube and mix gently. A vortex mixer may be used if available.
7. Incubate the mixtures at room temperature.
8. 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).
9. Once the RLU/s value is recorded on the screen open the drawer and discard the tube.
10. Determine luminescence for the remaining tubes.

CALCULATION OF RESULTS

1. Cut and paste the RLU/s values into the RLU/s column of the downloaded Excel spreadsheet.
2. CRP values (μg/ml) are automatically calculated.

TYPICAL RESULTS

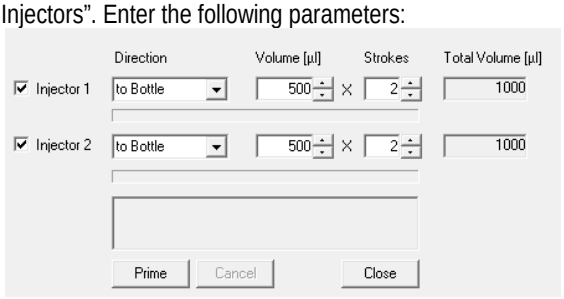
Typical results for the 0 and 10 ng/ml standards and six rabbit serum samples are shown below.

Rabbit CRP SIRIUS Worksheet						
Tube	Sample ID	Comments	Dilution	RLU/s	Zero subtracted RLU/s	ug/ml
1	0 ng/ml CRP			2945	0	
2	10 ng/ml CRP			80904	77959	
3	1	Healthy #2	200	19780	16835	0.43
4	2	Healthy #3	200	31858	28913	0.74
5	3	Healthy #10	200	12645	9700	0.25
6	4	Acute phase #179	16000	54338	51393	105.5
7	5	Acute phase #181	16000	64668	61723	126.7
8	6	Acute phase #182	16000	52770	49825	102.3

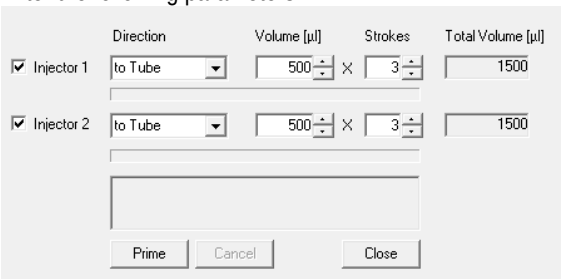
LUMINOMETER MAINTENANCE

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

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



2. 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.
3. Place the tubing from injectors one and two into separate tubes containing distilled or deionized water.
4. Enter the following parameters:



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

REFERENCES

1. Cray C, Rodriguez M and Fernandez Y. Acute phase protein levels in rabbits with suspected Encephalitozoon cuniculi infection. J. Exotic Pet Medicine. 22:280-286 (2013)
2. Destexhe E. et. al. Evaluation of C-reactive protein as an inflammatory biomarker in rabbits for vaccine nonclinical safety studies. J. Pharmacological and Toxicological Methods. 68:367-373 (2013)

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[2] 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.