# Dolphin SAA Sirius SPARCL Kit, Cat. No. SAA-SPL-18

## INTRODUCTION

SAA (serum amyloid A) is a positive acute phase reactant that is expressed in the liver and circulates in blood. Levels increase in response to infection, tissue injury and inflammation. The Sirius SPARCL™[1] assay allows rapid measurement of dolphin SAA using a Berthold Sirius-L single tube luminometer. Using the assay, we found SAA levels of 1 to 5 μg/ml in serum of healthy dolphins. Levels as high as 200 μg/ml were found in serum from sick dolphins.

## PRINCIPLE OF THE ASSAY

The assay uses two affinity purified dolphin SAA antibodies. One is conjugated to horseradish peroxidase (HRP), the other to acridan, a chemiluminescent substrate. When HRP and acridan conjugated antibodies bind to SAA 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 SAA concentration.

Diluted 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 SAA is determined from the ratio of sample luminescence to the that of a dolphin SAA standard.

## MATERIALS AND COMPONENTS

Materials provided with the kit:

- Acridan & HRP conjugates, 1 vial Store at -20°C
- Dolphin SAA 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 & 200 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)

⇩

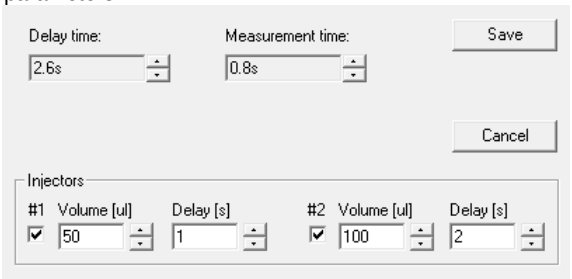
Incubate tubes at room temp for 45 min

⇩

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.

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

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## ASSAY FORMAT

1. Download the Excel spreadsheet from the Life Diagnostics website: https://lifediagnostics.com/dolphin-saa-spreadsheet/
2. The first two rows/tubes are reserved for the zero and 200 ng/ml standards.
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 SAA can range from 1 to 200 μg/ml, we recommend that samples be tested at a minimum of two dilutions. We suggest dilutions of 100 and 1000-fold.

1. For each sample dispense 495 μl and 360 μl of CSD50-1 diluent into separate tubes.
2. Mix 5.0 μl of serum with 495 μl of diluent to give a 100-fold dilution.
3. Mix 40.0 μl of the 100-fold diluted sample with 360 μl of diluent to give a 1000-fold dilution.

## STANDARD PREPARATION

1. Reconstitute the 200 ng/ml SAA standard with the volume of CSD50-1 diluent indicated on the vial label.

## CONJUGATE MIX PREPARATION

1. The acridan and HRP conjugate mix should be prepared just before use (step 6 in the Procedure section).
2. Tap the vial to ensure that the contents are at the bottom of the vial before carefully removing the stopper.
3. Add 5.5 ml of diluent CSD50-1 to the vial. Insert the stopper and mix gently by inversion 20x.
4. Each vial of reconstituted conjugate mix provides enough reagent to measure 0 and 200 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 200 ng/ml SAA 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. SAA values (μg/ml) are automatically calculated.

## TYPICAL RESULTS

Typical results for the 0 and 200 ng/ml standards and eight dolphin serum samples are shown below.

| Dolphin SAA SIRIUS Worksheet | | | | | | |
|---|---|---|---|---|---|---|
| Tube | Sample ID | Comments | Dilution | RLU/s | Zero subtracted RLU/s | ug/ml |
| 1 | 0 ng/ml SAA | | | 1186 | 0 | |
| 2 | 200 ng/ml SAA | | | 94063 | 92877 | |
| 3 | 1 | | 100 | 25257 | 24071 | 5.18 |
| 4 | 2 | | 100 | 4817 | 3631 | 0.78 |
| 5 | 3 | | 100 | 5518 | 4332 | 0.93 |
| 6 | 4 | | 100 | 3976 | 2790 | 0.60 |
| 7 | 5 | | 100 | 17048 | 15862 | 3.42 |
| 8 | 6 | | 100 | 24282 | 23096 | 4.97 |
| 9 | 7 | | 100 | 23684 | 22498 | 4.84 |
| 10 | 8 | | 1000 | 82529 | 81343 | 175.16 |

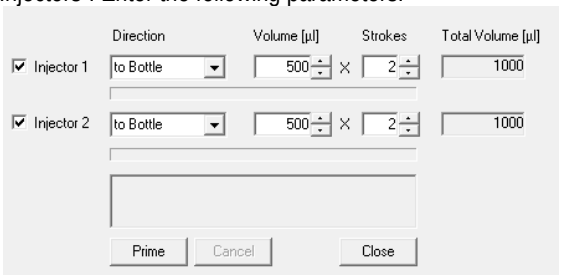
## PRECAUTIONS

The dolphin SAA assay does not achieve equilibrium within the 45-minute incubation period. RLU/s values increase gradually beyond 45-minutes. Luminescence of standards and samples should be measured quickly. No more than 18 samples should be tested with each set of zero and 200 ng/ml standards.

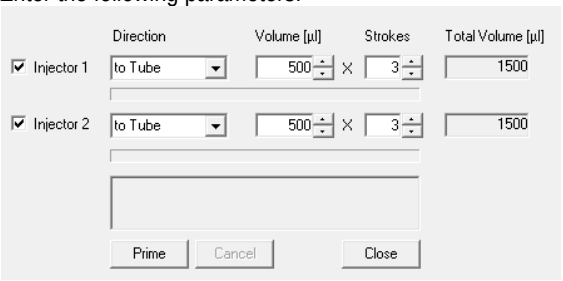
## LUMINOMETER MAINTENANCE

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.

[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. Please refer to the Precautions section.