Cat Cardiac Troponin-I Sirius SPARCL Kit, Cat. No. CTNI-SPL-8

INTRODUCTION

After cardiac injury, troponin-I (cTnI), is released into the blood from damaged muscle cells. Measurement of serum cTnI allows assessment of the extent of cardiac injury. It is widely used as a biomarker of heart damage in preclinical and veterinary research. The cat cTnI Sirius SPARCL™[1] assay allows rapid measurement of serum cTnI levels using a Berthold Sirius-L single tube luminometer.

PRINCIPLE OF THE ASSAY

The assay uses two cTnI-specific antibodies. One conjugated to horseradish peroxidase (HRP), the other to acridan, a chemiluminescent substrate. When HRP and acridan conjugated antibodies bind to cTnI 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 cTnI 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 automatic injection of background reducer, that eliminates nonspecific luminescence, followed by trigger-solution containing hydrogen peroxide. The concentration of cTnI is calculated from the ratio of sample luminescence to the luminescence of a cTnI standard.

MATERIALS AND COMPONENTS

Materials provided with the kit:

- Acridan & HRP conjugates, 1 vial Store at -20°C
- Cat cTnI standard, 1 vial Store at -20°C
- Conjugate diluent; CSD10-1, 10 ml
- Standard/sample diluent; SYD10-1, 10 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 rack
- 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 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)

⇩

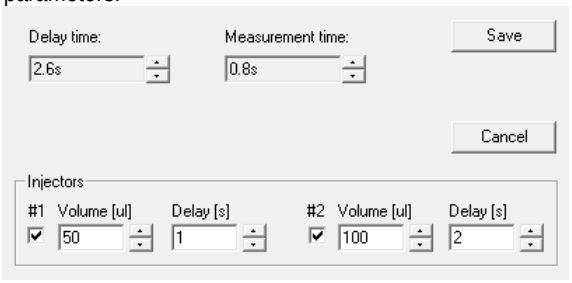
Incubate tubes at room temp for 45 min

⇩

Measure luminescence

LUMINOMETER SETUP

1. Switch the Sirius-L luminometer on and allow it to warm up for at least 15 minutes.
2. Place the tubing from injector one into a 5 ml centrifuge tube containing background reducer.
3. Place the tube from injector two into a 5 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/cat-ctni-spreadsheet/
2. The first two rows/tubes are reserved for the zero and 10 ng/ml standards.
3. 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

This assay is designed to use serum. Plasma cannot be used. Samples should be clear and non-hemolyzed. They must be diluted at least two-fold prior to assay by mixing one volume of serum with one volume of yellow standard/sample diluent (SYD-10-1). We typically mix 100 µl of serum with 100 µl of SYD10-1. If samples test out of range of the 10 ng/ml standard and further dilution is necessary, use SYD10-1.

STANDARD PREPARATION

1. Reconstitute the 10 ng/ml cTnI standard with the volume of yellow SYD10-1 diluent indicated on the vial label.
2. 100 µl 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 CSD10-1 (colorless) to the vial that contains the freeze-dried conjugates and mix gently by inversion 20x.
3. Each vial of reconstituted conjugate mix provides enough 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 yellow SYD10-1 diluent into tube one. This serves as the zero standard.
4. Pipet 100 µl of the reconstituted 10 ng/ml cTnI standard into tube two.
5. Pipet 100 µl aliquots of the diluted serum 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 determined by the luminometer into the RLU/s column of the downloaded Excel spreadsheet.
2. cTnI values are automatically calculated.

TYPICAL RESULTS

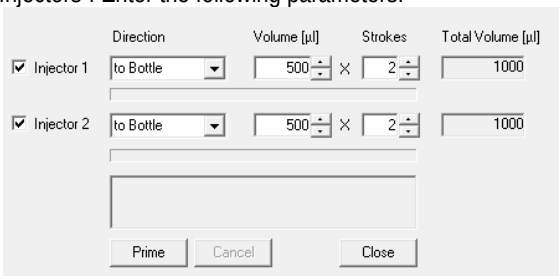
Typical results for the 0 and 10 ng/ml standards and two cat serum samples tested at a series of dilutions are shown below.

Cat cTnI SIRIUS Worksheet						
Tube	Sample ID	Comments	Dilution	RLU/s	Zero subtracted RLU/s	ng/ml
1	0 ng/ml cTnI			1446	0	
2	10 ng/ml cTnI			80752	79306	
3	Serum 12		32	5018	3572	14.41
4	Serum 12		16	8566	7120	14.36
5	Serum 12		8	17605	16159	16.30
6	Serum 12		4	32608	31162	15.72
7	Serum 12		2	63306	61860	15.60
8	Serum 36		128	3433	1987	32.07
9	Serum 36		64	5554	4108	33.15
10	Serum 36		32	8848	7402	29.87
11	Serum 36		16	16941	15495	31.26
12	Serum 36		8	32682	31236	31.51

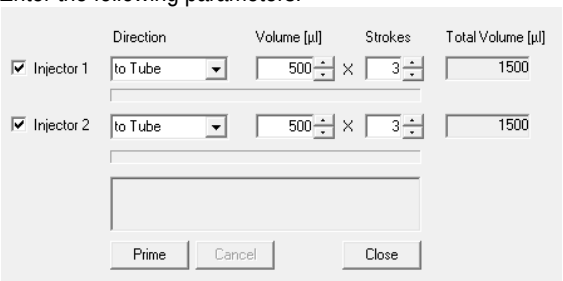
LUMINOMETER MAINTENANCE

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.

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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.