

RAT CARDIAC TROPONIN-I SPARCL™ ASSAY

Life Diagnostics, Inc., Catalog Number: CTNI-SP-2

INTRODUCTION

Troponin complex regulates striated muscle contraction. It is comprised of three subunits: troponin I, troponin C, and troponin T. Troponin I exists in three isoforms; one in fast-twitch, one in slow-twitch, and one in cardiac muscle. Cardiac troponin-I (cTnI) is significantly different from the skeletal muscle isoforms, allowing the development of cTnI specific immunoassays.

After cardiac injury, 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 cardiac biomarker in preclinical and veterinary research.

PRINCIPLE OF THE ASSAY

The rat cTnI SPARCL™¹ (Spatial Proximity Analyte Reagent Capture Luminescence, ref 1) assay uses two different affinity purified cTnI-specific antibodies that have been used in our preclinical cTnI ELISA kits² since 2006. One is conjugated to horseradish peroxidase (HRP) and the other is conjugated to acridan, a chemiluminescent substrate. When HRP and acridan conjugated cTnI antibodies bind to cTnI 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 determination of cTnI concentrations.

The HRP and acridan conjugated antibodies provided with the kit are mixed with standards and diluted samples in wells of the 96-well white SPARCL™ plate provided with the kit³. 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 cTnI is proportional to luminescence and is derived from a standard curve.

MATERIALS AND COMPONENTS

Materials provided with the kit:

- Anti-rat cTnI HRP conjugate stock. **Store ≤ -70°C**
- Anti-rat cTnI acridan conjugate stock. **Store ≤ -70°C**
- Rat cTnI stock (1 vial). **Store ≤ -20°C**
- Sample diluent (YD25-1)
- Conjugate diluent (CSD10-1)
- Trigger solution (TS11-1)
- White SPARCL™ plate (12 x 8-well)
- Clear untreated 96-well plate

Materials required but not provided:

- Precision pipettes, multichannel pipettors and tips
- Polypropylene tubes
- Vortex mixer
- Micro-Plate incubator/shaker
- Luminometer capable of simultaneous injection & measurement
- PC graphing software

STORAGE

Store the HRP conjugate, acridan conjugate at or below -70°C and the cTnI stock at or below -20°C. The remainder of the kit should be stored at 2-8°C. The SPARCL™ plate should be kept in a sealed bag with desiccant and antioxidant. The kit will remain stable for at least six months from the date of purchase, provided that the components are stored as described.

GENERAL INSTRUCTIONS

1. Please take the time to completely read all of the instructions before starting your assay. Contact us if you need clarification.
2. All reagents used in the assay should be allowed to reach room temperature (25°C) before use.
3. It is important that standards and samples be added to the SPARCL™ plate quickly. If testing a large number of samples, rather than pipetting standards and samples directly into the white SPARCL™ plate using a single channel pipettor, we recommend the following: First pipette an excess volume of standards and samples into appropriate wells of the clear 96-well plate. Then use an 8- or 12-channel multipipettor to quickly and efficiently transfer 80 µl aliquots to the appropriate wells of the white SPARCL™ plate. The wells of the clear plate have a maximum volume of 300 µl.

STANDARD PREPARATION

The lyophilized rat cTnI stock is comprised of stabilized rat cTnI diluted in a serum matrix. The cTnI content was determined by reference to purified rat cTnI prepared at Life Diagnostics, Inc.

1. Reconstitute the lyophilized stock with deionized or distilled water as described on the vial label. Mix gently until dissolved.
2. Label 8 polypropylene tubes as 5.0, 2.5, 1.25, 0.625, 0.313, 0.156, 0.078 and 0.039 ng/ml.
3. Into the tube labeled 5.0 ng/ml, pipette the volume of diluent detailed on the stock vial label. Then add the indicated volume of stock and mix gently. This provides the 5.0 ng/ml standard.
4. Dispense 250 µl of diluent into the tubes labeled 2.5, 1.25, 0.625, 0.313, 0.156, 0.078 and 0.039 ng/ml.
5. Pipette 250 µl of the 5 ng/ml cTnI standard into the tube labeled 2.5 ng/ml and mix. This provides the 2.5 ng/ml cTnI standard.
6. Similarly prepare the remaining standards by two-fold serial dilution.

Please Note: Use the standards within 30 minutes of preparation. If future use of the reconstituted cTnI stock is intended, it should be aliquoted and stored frozen at or below minus 20°C.

SAMPLE PREPARATION

Serum or heparin plasma should be prepared as quickly as possible after blood collection. EDTA plasma cannot be used in this assay because EDTA inhibits HRP. All samples should be similarly processed (i.e., storage times and temperatures should be the

¹ The SPARCL™ technology was developed by Lumigen Corp.

² A list of literature references citing use of our cTnI ELISA kits may be found on our website on the "Cardiac Biomarker ELISA's" page.

³ The white SPARCL™ plate provided with the kit has been treated with a reagent that reduces background chemiluminescence. Untreated plates cannot be used.

same). If serum samples cannot be assayed immediately they should be frozen at -70°C and thawed only once prior to use.

The levels of cTnI depend on the degree of cardiac injury and the time that blood is collected after injury. Optimal dilution factors must therefore be determined empirically. Serum can be tested undiluted. If dilution is necessary, use the sample diluent (YD25-1) provided with the kit (do not substitute other diluents).

CONJUGATE MIX PREPARATION

Instructions for preparation of the conjugate mix are detailed on the box that contains the HRP and acridan conjugates. Prepare the mix shortly before it is required using the conjugate diluent (CSD10-1) for dilution.

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 at least 1 ml of trigger solution.
3. Place the injection needle into the injection port, (necessary for BMG luminometers).
4. Program the luminometer to inject $75\ \mu\text{l}$ of trigger solution per well and to measure from time zero for 1 second (50×0.02 second intervals).
5. Define the format of the assay using the luminometer software.
6. Because the white SPARCL™ plate is provided as a 12×8 -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 LUMIstar 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 $2-8^{\circ}\text{C}$.
3. Aliquot $20.0\ \mu\text{l}$ of conjugate mix into each well.
4. Dispense $80.0\ \mu\text{l}$ 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 ($75\ \mu\text{l}$).
7. Remove the plate from the luminometer and discard the used strips. Keep the plate frame if future use is intended.

CALCULATION OF RESULTS

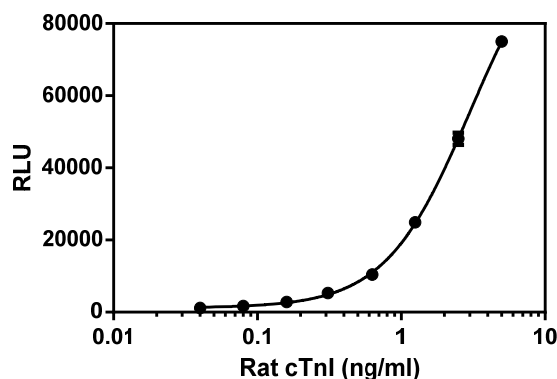
1. Before calculating results, review the raw data. If artefacts (RLU spikes) are apparent immediately after injection of trigger solution, eliminate that portion of the luminescence profile from analysis for all wells.
2. Using graphing software, construct a standard curve by plotting the luminescence (RLU) for the standards versus Log_{10} cTnI concentration.

3. Fit the data using a four parameter dose response model.
4. Derive the corresponding concentration of cTnI in the samples from the standard curve (derive the antilog of the log_{10} value).
5. Multiply the derived concentration by the dilution factor to determine the actual concentration of cTnI in the serum 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 log_{10} cTnI 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. A standard curve must be run with each experiment.

cTnI (ng/ml)	RLU
5.0	75000
2.5	48072
1.25	24907
0.625	10445
0.313	5290
0.156	2816
0.078	1687
0.039	1192



REFERENCES

1. Akhavan-Tafti H. et al. A homogeneous chemiluminescent immunoassay method. *J Am Chem Soc.* 20;135(11):4191-4 (2013)

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