RABBIT CARDIAC TROPONIN-I SPARCL™ ASSAY
Life Diagnostics, Inc., Catalog Number: CTNI-SP-10

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 in preclinical and veterinary research.

PRINCIPLE OF THE ASSAY

The rabbit 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 kits[2] 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 kit[3]. 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-cTnI HRP stock **Store ≤ -70°C**
- Anti-cTnI acridan stock **Store ≤ -70°C**
- cTnI stock **Store ≤ -20°C**
- Sample diluent; YD25-1, 25 ml
- Conjugate diluent; CSD10-1, 10 ml
- Trigger solution; TS11-1, 11 ml
- White SPARCL™ plate (12 x 8-well)
- Clear untreated 96-well plate

Materials required but not provided:

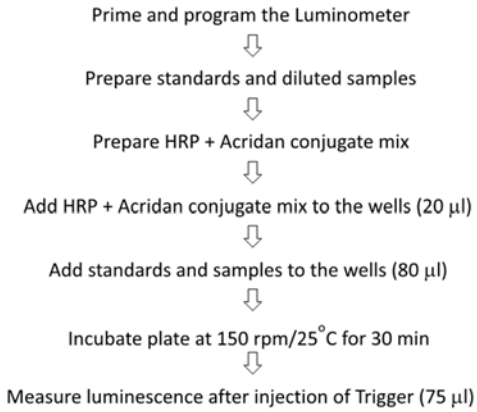
- Precision pipettes and tips
- Polypropylene microcentrifuge tubes
- Vortex mixer
- Plate incubator/shaker
- Luminometer capable of simultaneous injection/measurement
- Curve fitting software

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 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 many 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 50 µl aliquots to the appropriate wells of the white SPARCL™ plate. The wells of the clear plate have a maximum volume of 300 µl.
4. Follow the sequence of events below when running the assay.



STANDARD PREPARATION

The lyophilized rabbit cTnI stock is comprised of stabilized rabbit cTnI diluted in a serum matrix. The cTnI content was determined by reference to purified rabbit 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 10.0, 5.0, 2.5, 1.25, 0.625, 0.313, 0.156 and 0.078 ng/ml.
3. Into the tube labeled 10.0 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 10.0 ng/ml standard.
4. Dispense 250 µl of diluent into the tubes labeled 5.0, 2.5, 1.25, 0.625, 0.313, 0.156 and 0.078 ng/ml.

[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.

Life Diagnostics, Inc., P.O. Box 5205, West Chester, PA 19380
610-431-7707 – 610-431-7818 (Fax)
info@lifediagnostics.com – www.lifediagnostics.com

5. Pipette 250 µl of the 10.0 ng/ml cTnI standard into the tube labeled 5.0 ng/ml and mix. This provides the 5.0 ng/ml cTnI standard.
6. Similarly prepare the remaining standards by two-fold serial dilution.

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 -20°C.

SAMPLE PREPARATION

Serum or heparin plasma should be prepared as quickly as possible after blood collection and stored at 4°C. 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 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 without matrix effects. If dilution is necessary, use the YD25-1 diluent (yellow solution) provided with the kit

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 µl of trigger solution per well and to measure from time zero for 1 second (50 x 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 x 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°C.
3. Aliquot 20.0 µl of conjugate mix into each well.
4. Dispense 80.0 µl of standards and diluted samples into the wells (standards and samples should be tested in duplicate).
5. Incubate on an orbital micro-plate shaker at 150 rpm and 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 µl).
7. Remove the plate from the luminometer and discard the used strips. Keep the plate frame if future use is intended.

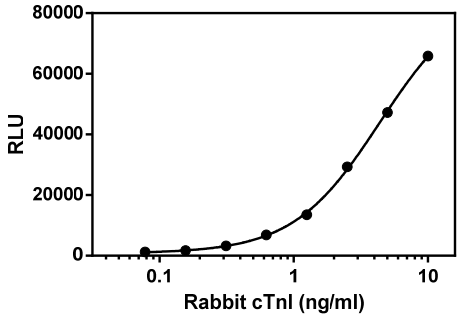
CALCULATION OF RESULTS

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. We routinely use the sum of RLU values from a 100-980 ms data collection window.
2. Determine the sum of RLU values within the data collection window for the standards and samples.
3. Using graphing software, construct a standard curve by plotting the sum of the RLU values for the standards versus the $\log_{10}$ of cTnI concentration and fit to a sigmoidal, 4PL model.
4. Derive the corresponding concentration of cTnI in the samples from the standard curve (remember to derive the concentration from the antilog).
5. Multiply the derived concentration by the dilution factor to determine the concentration of cTnI in the original sample.
6. If the sum of the RLU values of diluted samples fall outside the standard curve, samples should be appropriately diluted and re-tested.

TYPICAL STANDARD CURVE

A typical standard curve is shown below. This curve is for illustration only and should not be used to calculate unknowns. A standard curve should be run with each experiment.

cTnI (ng/ml)	RLU
10.0	65858
5.0	47260
2.5	29330
1.25	13504
0.625	6832
0.313	3227
0.156	1725
0.078	1211



REFERENCES

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

Rev 022118

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