SKELETAL MUSCLE TROPONIN-C SPARCL™ ASSAY
Life Diagnostics, Inc., Catalog Number: STNC-SP

FOR RESEARCH USE ONLY. NOT FOR USE WITH HUMAN SAMPLES IN CLINICAL DIAGNOSTIC PROCEDURES.

INTRODUCTION

The troponin complex regulates contraction of striated muscle. It is a heterotrimer of three polypeptides; troponin-I, troponin-C, and troponin-T. Two troponin-C (TnC) isoforms are expressed; one in fast-twitch skeletal muscle and one in cardiac and slow-twitch skeletal muscle. Antibodies used in the kit specifically recognize the fast-twitch TnC isoform. Fast-twitch TnC is highly conserved across species; reactivity with fast-twitch TnC from other species is highly likely. Thus far, we have confirmed reactivity with mouse, rat, rabbit, pig, goat, cow and chicken.

TnC is significantly more stable in serum than skeletal muscle troponin-I making it a more useful biomarker than troponin-I for assessment of skeletal muscle injury.

PRINCIPLE OF THE ASSAY

The STNC SPARCL™[1] (Spatial Proximity Analyte Reagent Capture Luminescence, ref 1) assay uses two different fast-twitch TnC specific antibodies. Mouse monoclonal anti-TnC (13E11-84-14) is conjugated to horse radish peroxidase (HRP). Affinity purified rabbit polyclonal anti-TnC is conjugated to acridan, a chemiluminescent substrate. When HRP and acridan conjugated antibodies bind to TnC 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 TnC concentrations.

The HRP and acridan conjugated antibodies provided with the kit are mixed with standards and diluted samples in wells of the 96-well SPARCL™ plate provided with the kit[2]. 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 TnC is proportional to luminescence and is derived from a standard curve.

MATERIALS AND COMPONENTS

Materials provided with the kit:

- Anti- TnC HRP conjugate stock. **Store ≤ -70°C**
- Anti- TnC acridan conjugate stock. **Store ≤ -70°C**
- TnC stock (3 vials). **Store ≤ -70°C**
- Diluent (CSD50-1)
- Trigger solution, 11 ml
- White SPARCL™ plate (12 x 8-well)
- Clear untreated 96-well plate

Materials required but not provided:

- Precision pipettes 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 and TnC stock at or below -70°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 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.

STANDARD PREPARATION

The lyophilized TnC stock is comprised of rabbit fast-twitch TnC lyophilized in a carrier protein matrix.

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 50, 25, 12.5, 6.25, 3.13, 1.56, 0.78 and 0.39 ng/ml.
3. Into the tube labeled 50 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 50 ng/ml standard.
4. Dispense 250 µl of diluent into the tubes labeled 25, 12.5, 6.25, 3.13, 1.56, 0.78 and 0.39 ng/ml.
5. Pipette 250 µl of the 50 ng/ml TnC standard into the tube labeled 25 ng/ml and mix. This provides the 25 ng/ml TnC standard.
6. Similarly prepare the remaining standards by two-fold serial dilution.

Please Note: Use the standards within 30 minutes of preparation.

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 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 TnC depend on the degree of skeletal muscle injury and the time that blood is collected after injury. Optimal dilution

[1] The SPARCL™ technology was developed by Lumigen Corp.

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

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factors must therefore be determined empirically. Serum samples may need to be diluted in order to avoid matrix effects. We found it necessary to dilute serum at least 8-fold in order to avoid matrix effects and false low values. Do not use diluents other than that 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 use using the conjugate diluent (CSD50-1) 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 as needed for BMG luminometers.
4. Program the luminometer to inject 37.5 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 25.0 µl of conjugate mix into each well.
4. Dispense 50.0 µl 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 (37.5 µl).
7. Remove the plate from the luminometer and discard the used strips. Keep the plate frame if future use is intended.

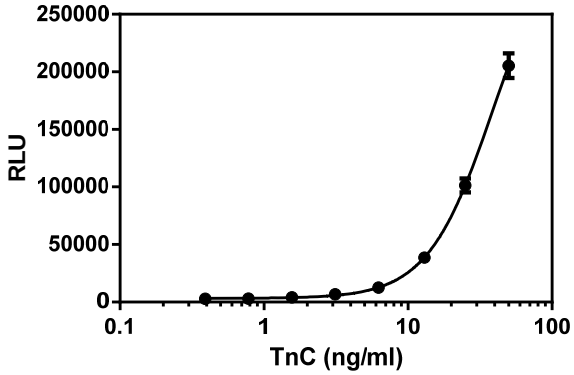
CALCULATION OF RESULTS

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.
2. Using graphing software, construct a standard curve by plotting the luminescence (RLU) for the standards versus the $\log_{10}$ of the TnC concentration.
3. Fit the data using a four parameter dose response model.
4. Derive the corresponding concentration of TnC 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 Multiply the derived concentration by the dilution factor to determine the actual concentration of TnC 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 TnC 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.

TnC (ng/ml)	RLU
50	205374
25	101405
12.5	38597
6.25	12563
3.13	6720
1.56	4052
0.78	3011
0.39	2767



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