INTRODUCTION

Troponin-C is a muscle protein that regulates contraction as part of the troponin ITC-complex. Two troponin-C isoforms are expressed, one in fast-twitch skeletal muscle and one in cardiac and slow-twitch skeletal muscle. This assay measures the fast-twitch troponin-C isoform (STNC).

The salmon STNC assay uses SPARCL™[1] technology. Two STNC antibody conjugates are used. One to horseradish peroxidase (HRP), the other to acridan; a chemiluminescent substrate. When HRP and acridan conjugates bind to STNC 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 STNC concentration.

In practice, a blank, a standard, and diluted samples (10 µl) are pipetted into test tubes and mixed with 0.5 ml of combined acridan and HRP conjugates. After 45 minutes, the tubes are sequentially inserted into the VetBio-1 luminometer. Luminescence is measured after injection of background reducer, followed by hydrogen peroxide containing trigger-solution. The concentration of STNC is determined from the ratio of blank-subtracted sample luminescence to the that of the standard.

Equilibrate reagents to room temperature
⇩
Prime the luminometer
⇩
Program the luminometer and save the program
⇩
Dilute samples
⇩
Prepare standard
⇩
Add blank, standard and diluted samples to tubes (10 µl/tube)
⇩
Reconstitute the acridan/HRP conjugate mix (5.5 ml/vial)
⇩
Add conjugate mix to the tubes (0.5 ml/tube)
⇩
Incubate tubes at room temp for 45 min
⇩
Run the Quick Prime program
⇩
Load the program. Test standards and samples

STORAGE

Store the conjugate and STNC standard vials at or below -20°C. The remainder of the kit should be stored at 4°C.

MATERIALS AND COMPONENTS

Materials provided with the kit:

- Acridan & HRP conjugates, 5 vials. **Store at -20°C**
- STNC standard, 5 vials. **Store at -20°C**
- Diluent: CSD50-1, 50 ml
- Background reducer: BR9-1, 9 ml
- Trigger solution: TS12-1, 12 ml

Materials required but not provided:

- VetBio-1 luminometer
- Precision pipettors and tips
- 12 x 75 mm borosilicate tubes[2]
- 12 x 75 mm test tube racks
- De-ionized water
- 15-ml centrifuge tubes & rack
- 10-ml pipets

[1] SPARCL technology, using acridan- and HRP-conjugated antibodies, was developed by and is licensed from Lumigen Corp.
[2] Only cylindrical 12 x 75 mm borosilicate glass tubes can be used. Do not use polystyrene or polycarbonate tubes.

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 allowed to reach room temperature before use.

LUMINOMETER SETUP

1. Turn the VetBio-1 luminometer on.
2. Place the tubing from injector 1 into the tube containing background reducer. Tube holders are positioned adjacent to the injectors.
3. Place the tube from injector 2 into the tube containing trigger solution.
4. From the Protocol manager on the keypad select “Prime & Wash”, "Prime", then “Start”.
5. Open the luminometer drawer and insert an empty 12 x 75 mm tube.
6. Close the drawer and click “Start” again.
7. Wait for priming to complete. Open the drawer and discard the tube.
8. From the Protocol manager select “Measure new experiment”” followed by “Dog Haptoglobin (Serum)” or “Dog Haptoglobin (Feces)”
9. Select “Start”.
10. The experiment setup screen will be displayed. Enter the number of samples you intend to measure, then press “OK”. Please note that the number of samples does not include the two tubes used for the blank and standard.
11. Enter the sample IDs and dilution factor(s), if different than the default dilution. You can also enter experimental comments.
12. After entering information, press save. The experiment will be saved as a “prepared experiment”.

SAMPLE PREPARATION

We found levels of STNC ranging from 0.2 μg/ml in healthy fish to >90 μg/ml in fish with pancreatic disease. Optimal dilutions should be determined empirically. However, we suggest that samples be tested initially at a dilution of 100-fold.

1. For each sample to be tested pipet 200 μl of CSD50-1 diluent into a microcentrifuge tube.
2. Add 2.0 ul of sample and mix. This provides a 100-fold dilution.
3. Use 10.0 ul as described in the procedure section.

STANDARD PREPARATION

1. The STNC standard is provided in lyophilized form.
2. Add the volume of CSD50-1 diluent indicated on the vial label and mix. Use a vortex mixer if available. This provides a 50 ng/ml standard.
3. Pipet 10.0 ul into tube 2 as described in procedure section.

Use the standard within 30 min of reconstitution.

CONJUGATE PREPARATION

1. The acridan and HRP conjugate mixture should be prepared just before use.
2. Tap the vial to ensure that the contents are at the bottom of the vial before slowly removing the stopper (it is under vacuum).
3. Add 5.5 ml of diluent CSD50-1 to the vial. Insert the stopper and mix gently by inversion several times.
4. Each vial of reconstituted conjugate mix provides enough reagent to measure the blank, standard, and up to eight samples.[3]

PROCEDURE

STEP 1

1. Determine the number of 12 x 75 mm borosilicate glass tubes required (one each for the blank, standard, and the samples). Ensure that all the tubes you intend to use fit easily in the VetBio-1 sample holder before beginning the test.
2. Pipet 10 μl of diluent into tube 1. This serves as the zero standard or blank.
3. Pipet 10 μl of the 50 ng/ml STNC standard into tube 2.
4. Pipet 10 μl of the diluted samples into tubes 3, 4, 5…
5. Add 0.50 ml of freshly prepared conjugate mix to each tube and mix gently. A vortex mixer may be used if available. Avoid creating bubbles.
6. Incubate the mixtures at room temperature for 45 minutes (incubations as short as 15-minutes can be used if testing eight or fewer samples).

STEP 2

1. Two to three minutes before the end of the incubation step, insert an empty tube into the sample holder. Select “QuickPrime” from the Protocol manager, close the drawer and press start. Discard the tube after completion. This step ensures that the injectors are fully loaded.
2. Recall your saved experiment and press start.
3. After the end of the incubation, insert tube 1 (Blank) into the sample holder of the luminometer and close the drawer. When the luminescence value is recorded on the screen open the drawer and remove the tube.
4. Similarly measure luminescence of the standard (tube 2), followed by the samples (tubes 3, 4, 5…).
5. Haptoglobin concentrations of the samples are automatically calculated.
6. After measurement of the last sample, select “End”.
7. Results will be saved and can be recalled. They may be exported as Excel or pdf files via a USB stick.

[3] 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.

RESULTS

If RLU/s values for diluted samples are between the blank and standard, assume that the calculated values are correct. If RLU/s values significantly exceed that of the standard, STNC concentrations may be underestimated. If an accurate measurement is required, such samples should be retested at a higher dilution.

LUMINOMETER MAINTENANCE

The luminometer injectors must be cleaned with distilled or deionized water at the end of each day that the instrument is used to avoid clogging.

1. From the Protocol manager screen select “Prime & Wash”.
2. Select “Backprime” to return unused reagents to the respective tubes.
3. Cap the background reducer and trigger solution tubes and store them at 4°C.
4. Wipe the tubing from injectors 1 and 2 and place them in separate 15 ml centrifuge tubes containing distilled or deionized water.
5. Select “Wash” from the “Prime & Wash” screen. Press “Start”, insert an empty tube into the luminometer, close the drawer and press “Start”.
6. Discard the tube.
7. Insert an empty tube and repeat steps 5 and 6.
8. Leave the injector tubing immersed in water.
9. Switch the luminometer off. It should not be left powered-up overnight.

REPLICATES

Replicate measurements of the blank/standard and samples can be measured. This requires minor editing of the program.

1. In Protocol manager, select “Measure new experiment” and the program you would like to edit.
2. Press “Edit”.
3. You can change the sample dilution factor, the number of replicates for the blank and standard, and the number of replicates for samples (leave the standard concentration as 50 ng/ml).
4. Press Start, enter the number of samples, and save the experiment.

Please note that these settings will be saved for future runs unless re-edited.

ASSAY PERFORMANCE

Typical data: The tables below show results from assays in which the zero standard (blank), 50 ng/ml standard, and eight serum samples were tested. The “m” samples were from healthy fish, the “pd” samples were from fish with pancreatic disease.

Veterinary Biomarkers, Inc.

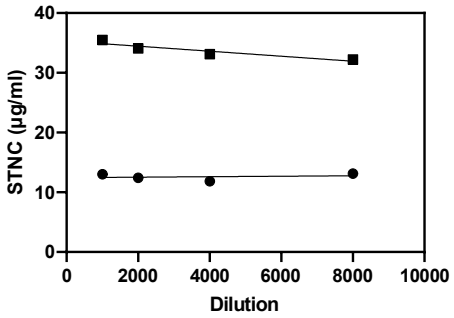
Salmon Skeletal TnC Exp#008 25/08/2021 13:49:32

Single Assay	Start by :	DOOR	Blank :	ON	
Measurement	Delay [s] :	2.600	Time [s] :	0.800	
Injector 1	Delay [s] :	1.000	Volume [µL] :	50	
Injector 2	Delay [s] :	2.000	Volume [µL] :	100	

Experiment comment: Healthy & PD fish

Sample	Dilution	Rep	RLU/s	Conc [µg/mL]
Blank			4,496	0
Standard			304,250	0.05
m5	100		15,534	0.18411
m6	100		10,249	0.095954
m7	100		20,418	0.26557
m8	100		8,705	0.070203
pd7	1000		406,691	67.088
pd8	1000		327,614	53.897
pd9	1000		496,355	82.044
pd10	1000		150,190	24.302

Linearity: To assess the linearity of the assay, two samples containing STNC at concentrations of 12.6, and 33.7 µg/ml were serially diluted to give values within range of the assay.



Rev 082521

Veterinary Biomarkers, Inc.,
124 Turner Lane, West Chester, PA 19380
484-200-9651 - info@vetbiomarkers.com – www.vetbiomarkers.com