SKELETAL MUSCLE TROPONIN-C ELISA
Life Diagnostics, Inc., Catalog Number: STNC

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. This ELISA specifically recognizes the fast-twitch skeletal muscle TnC isoform (F-TnC). It can be used to investigate skeletal muscle injury. Antibodies used in the kit were generated against rabbit F-TnC. F-TnC is highly conserved across species; reactivity with F-TnC from other species is highly likely. Thus far, we have directly confirmed reactivity with F-TnC from mouse, rat, rabbit, dog, pig, goat, cow, and chicken.

Advantageously, F-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

F-TnC antibody is used for capture, immobilized on the microtiter wells and an HRP conjugated F-TnC antibody is used for detection. Standards and diluted serum samples (100 µl/well) are incubated in the microtiter wells for 45 minutes. The wells are then washed. Diluted HRP conjugate (100 µl/well) is added to the wells and incubated for 45 minutes. F-TnC molecules, if present, are sandwiched between the capture and detection antibodies. After washing the wells to remove unbound HRP-conjugate, TMB (100 µl/well) is added and incubated for 20 minutes. If F-TnC is present a blue color develops. Color development is stopped by addition of stop solution (100 µl/well), changing the color to yellow. Absorbance at 450 nm is then measured. The concentration of F-TnC is proportional to the absorbance and is derived from a standard curve.

MATERIALS AND COMPONENTS

Reagents and materials provided:

- Anti-skeletal muscle TnC coated wells (12 x 8-well strips)
- F-TnC stock, 1 vial
- Diluent: CSDT50-1, 50 ml
- 20X Wash Solution: TBS50-20, 50 ml
- HRP conjugate stock, 1 vial
- TMB reagent: TMB11-1, 11 ml
- Stop solution: SS11-1, 11 ml

Materials required but not provided:

- Distilled or deionized water
- Pipettes
- Plate reader capable of reading at 450 nm
- Vortex mixer
- Absorbent paper
- PC graphing software or graph paper
- Polypropylene microcentrifuge tubes (1.5 ml)
- Plate shaker/incubator with a mixing speed of 150 rpm

STORAGE

The unused kit should be stored at 4°C and the microtiter plate should be kept in a sealed bag with desiccant. Kits will remain stable for six months from the date of purchase.

GENERAL INSTRUCTIONS

1. All reagents should be allowed to reach room temperature before use.
2. Reliable and reproducible results will be obtained when the assay is carried out with a complete understanding of the instructions and with adherence to good laboratory practice.
3. The wash procedure is critical. Insufficient washing will result in poor precision and falsely elevated absorbance readings.
4. Laboratory temperature will influence absorbance readings. Our ELISA kits are calibrated using shaking incubators set at 150 rpm and 25°C. Performance of the assay at lower temperatures will result in lower absorbance values.

WASH SOLUTION PREPARATION

The wash solution is provided as a 20x stock. Prior to use dilute the contents of the bottle (50 ml) with 950 ml of distilled or deionized water.

STANDARD PREPARATION

1. Reconstitute the lyophilized F-TnC stock as detailed on the vial label.
2. Label eight polypropylene tubes as 10, 5, 2.5, 1.25, 0.625, 0.313, 0.156 and 0 ng/ml.
3. In the tube labeled 10 ng/ml prepare the 10 ng/ml standard as detailed on the stock vial label.
4. Pipette 0.25 ml of diluent into the tubes labeled 5, 2.5, 1.25, 0.625, 0.313, 0.156 and 0 ng/ml.
5. Prepare a 5 ng/ml standard by diluting and mixing 0.25 ml of the 10 ng/ml standard with 0.25 ml of diluent in the tube labeled 5 ng/ml.
6. Similarly prepare the 2.5 to 0.156 ng/ml standards by serial dilution.
7. Reconstituted F-TnC stock is stable for several hours at room temperature but we recommend that unused stock be immediately frozen at or below -20°C if future use is intended.

SAMPLE PREPARATION

Because F-TnC levels depend on the degree of muscle damage optimal sample dilutions must be determined empirically. However, to avoid matrix effects and false low values, samples should be diluted at least 10-fold with the diluent (CSDT50-1). Do not substitute other diluents.

ASSAY PROCEDURE

1. Secure the desired number of 8-well strips in the holder. Unused strips should be stored in the re-sealed bag with desiccant at 4°C for future use.
2. Dispense 100 µl of standards and diluted samples into the wells (we recommend that samples and standards be run in duplicate).
3. Incubate on an orbital micro-plate shaker at 150 rpm and 25°C for one hour.
4. Empty and wash the microtiter wells 5x with 1x wash solution using a plate washer (400 µl/well).
5. Strike the wells sharply onto absorbent paper or paper towels to remove all residual droplets.

6. Add 100 µl of diluted HRP conjugate to each well.
7. Incubate on an orbital micro-plate shaker at 150 rpm and 25°C for 45 minutes.
8. Wash as described in steps 4 and 5 above.
9. Dispense 100 µl of TMB into each well.
10. Incubate on an orbital micro-plate shaker at 150 rpm at 25°C for 20 minutes.
11. After 20-minutes, stop the reaction by adding 100 µl of Stop solution to each well.
12. Gently mix. It is important to make sure that all the blue color changes to yellow.
13. Read absorbance at 450 nm with a plate reader within 5 minutes.

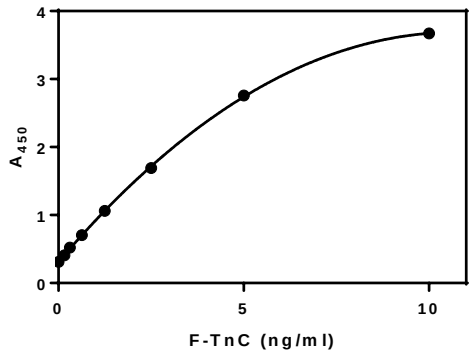
CALCULATION OF RESULTS

1. Using curve fitting software, construct a standard curve by plotting absorbance values of the standards versus the concentration.
2. Fit the standard curve to an appropriate model (we use a two site, total and non-specific binding model) and determine the concentration of the samples from the standard curve.
3. Multiply the derived concentration by the dilution factor to determine the actual concentration in the plasma sample.
4. If the A_{450} values of samples fall outside the standard curve, samples should be diluted appropriately 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. Each user should obtain his or her data and standard curve in each experiment.

F-TnC (ng/ml)	Absorbance (450 nm)
10	3.670
5	2.757
2.5	1.691
1.25	1.060
0.625	0.706
0.313	0.520
0.156	0.407
0	0.312



SPECIFICITY

1. Cardiac troponin-C and cardiac troponin-ITC complex showed no reactivity in the assay when tested at concentrations of one µg/ml.
2. The assay recognizes free F-TnC and TnC complexed with skeletal muscle troponin-I and troponin-T.

Rev 010221

For technical assistance please email us at
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