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

Skeletal Muscle Troponin-C ELISA

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

BACKGROUND

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, pig, goat, cow and chicken.

Advantageously, F-TnC is significantly more stable in serum than skeletal muscle troponin-I making it a potentially 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 or plasma 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 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, 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 (1 plate, 96 wells)
- F-TnC stock (1 vial)
- Diluent (CSDT50-1, 50 ml)
- Wash buffer (TBS50-20, 20x stock, 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

Store the kit at 2-8°C. Keep the microtiter plate in a sealed bag with desiccant.

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 8 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.

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, in order to avoid matrix effects and false low values, samples should be diluted at least 10-fold with the diluent (CSD50-1). Do not substitute other diluents.

HRP CONJUGATE PREPARATION

The HRP conjugate is provided as a concentrated stock that must be diluted with diluent (CSD50-1). Determine the number of 8-well strips to be used and prepare the working conjugate solution as detailed on the stock vial label.

ASSAY PROCEDURE

1. Secure the desired number of 8-well strips in the cassette.
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[1] for one hour.
4. Wash and empty the microtiter wells six times with wash solution using a plate washer (400 µl/well). The entire wash procedure should be performed as quickly as possible.
5. Strike the wells sharply onto absorbent paper or paper towels to remove residual wash solution.

[1]This ELISA was validated using a shaking incubator set at 150 rpm and 25°C. Performance of the assay at lower temperatures will result in lower absorbance values but will not invalidate the assay.

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 the wells as detailed in steps 4 and 5 above.
9. Dispense 100 µl of TMB reagent into each well.
10. Gently mix on an orbital micro-plate shaker at 150 rpm at 25°C for 20 minutes.
11. 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 the optical density at 450 nm with a microtiter plate reader *within 5 minutes*.
14. If absorbance values of samples exceed that of the 10 ng/ml standard, samples should be further diluted and re-tested.

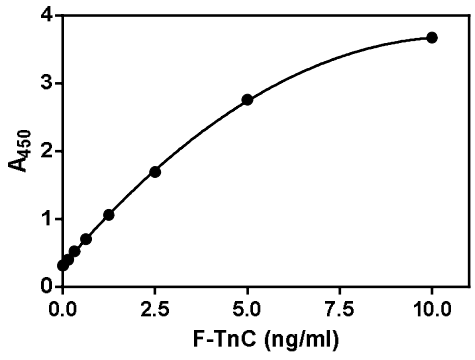
CALCULATION OF RESULTS

1. Calculate the mean absorbance value (A_{450}) for the standards and samples.
2. Construct a standard curve by plotting the A_{450} values obtained for each reference standard against its concentration in ng/ml on graph paper, with absorbance on the vertical (y) axis and concentration on the horizontal (x) axis.
3. Using the A_{450} values for each sample, determine the corresponding concentration of F-TnC (ng/ml) from the standard curve. If available, graphing software should be used. We suggest fitting the standard curve to a single site – total and nonspecific binding equation.
5. Multiply the derived F-TnC concentration by the dilution factor to obtain the actual F-TnC concentration.

TYPICAL STANDARD CURVE

Results of a typical standard curve with A_{450} plotted on the Y axis against F-TnC concentrations on the X axis are shown below. **NOTE:** This standard curve is for the purpose of illustration only.

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



PROCEDURAL NOTES

1. Standards should be prepared just before use.
2. HRP conjugate should be prepared just before use.
3. The dilution buffer supplied with the kit (CSD50-1) must be used for dilution of standards, samples and HRP conjugate. Do not substitute other buffers.
4. We recommend that standards and samples be run in duplicate.
5. Reliable and reproducible results will be obtained when the assay procedure is carried out with a complete understanding of the kit instructions. Please read and fully understand the instructions before starting.
6. Insufficient washing will result in poor precision and falsely elevated absorbance readings.

SPECIFICITY

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

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For technical assistance please email us at
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