RAT SKELETAL MUSCLE TROPONIN-I ELISA KIT
Life Diagnostics, Inc., Catalog Number: STNI-2

Rat Skeletal Muscle Troponin-I (SkM-TnI) ELISA

BACKGROUND

Troponin is the contractile regulating protein complex of striated muscle. It consists of three polypeptides: troponin-I, troponin-C, and troponin-T. Three troponin-I isoforms are expressed: one each in fast-twitch and slow-twitch skeletal muscle fibers and one in cardiac muscle. Following muscle injury troponin-I is released into the blood. It therefore serves as a good biomarker of muscle injury. This ELISA kit is specific for the fast twitch isoform of troponin-I and can be used to assess skeletal muscle damage.

PRINCIPLE OF THE ASSAY

The assay uses two different antibodies. A polyclonal SkM-TnI antibody is used for capture, immobilized on the microtiter wells. An HRP conjugated monoclonal antibody specific for fast twitch SkM-TnI is used for detection. Samples and HRP conjugate are co-incubated in the microtiter wells[1] for one hour. SkM-TnI molecules, if present, are sandwiched between the immobilization and detection antibodies. The wells are then washed to remove unbound HRP-conjugate; TMB is added and incubated for 20 minutes. If troponin-I 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 troponin-I is proportional to the absorbance and is derived from a standard curve.

MATERIALS AND COMPONENTS

Reagents and materials provided:

- Anti SkM-TnI coated wells (1 plate, 96 wells)
- SkM-TnI stock (3 vials)
- Diluent: TNID50-1, 50 ml
- 20X Wash buffer: TBS50-20, 50 ml
- HRP conjugate stock (50 µl)
- 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 SkM-TnI stock vials and the HRP conjugate stock at or below -20°C. Store the remainder of 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 one vial of the lyophilized SkM-TnI stock as detailed on the vial label.
2. Label 7 polypropylene tubes as 20, 10, 5, 2.5, 1.25, 0.625, and 0.313 ng/ml.
3. In the tube labeled 20 ng/ml prepare the 20 ng/ml standard as detailed on the stock vial label.
4. Pipette 0.25 ml of diluent into the tubes labeled 10, 5, 2.5, 1.25, 0.625 and 0.313 ng/ml.
5. Prepare a 10 ng/ml standard by diluting and mixing 0.25 ml of the 20 ng/ml standard with 0.25 ml of diluent in the tube labeled 10 ng/ml.
6. Similarly prepare the 5 to 0.313 ng/ml standards by serial dilution.

NOTE: The reconstituted SkM-TnI standards should be used within 30 minutes of stock reconstitution. Discard the stock after use.

SAMPLE COLLECTION

Serum or plasma should be prepared as quickly as possible after blood collection. All samples should be similarly processed (i.e., storage times and temperatures should be the same). If samples cannot be assayed immediately, they should be aliquoted and frozen at –70°C. Multiple freeze-thaws must be avoided.

SAMPLE PREPARATION

In studies at Life Diagnostics, Inc., we have encountered samples with very low (≤ 4 ng/ml) and high (> 500 ng/ml) levels of SkM-TnI. The optimal dilution of samples must be determined empirically. To avoid matrix effects and false low values, we recommend that samples be diluted at least 10-fold with diluent. If, at the discretion of the researcher, samples are tested at lower dilutions (i.e., 5-fold), the same dilution factor must be used for all samples.

HRP CONJUGATE PREPARATION

The HRP conjugate is provided as a concentrated stock. 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. 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.
3. Strike the wells sharply onto absorbent paper or paper towels to remove any residual wash solution. The wells must not be allowed to dry prior to addition of standards and samples.[2]
4. Dispense 100 µl of standards and diluted samples into the wells (we recommend that samples and standards be run in duplicate).

[1]Please note that the microtiter strips must be washed as described in the "Assay Procedure" section prior to use.

[2]Prior to washing the coated wells, we prepare or dispense at least 140 µl of standards and samples into appropriate wells of a blank polystyrene plate. This allows rapid transfer of 100 µl aliquots into the assay plate using a multipipettor.

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5. Add 100 µl of diluted HRP conjugate to each well.
6. Incubate on an orbital micro-plate shaker at 150 rpm and 25°C[3] for one hour.
7. 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.
8. Strike the wells sharply onto absorbent paper or paper towels to remove residual wash solution.
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 20 ng/ml standard, samples should be further diluted and re-tested.

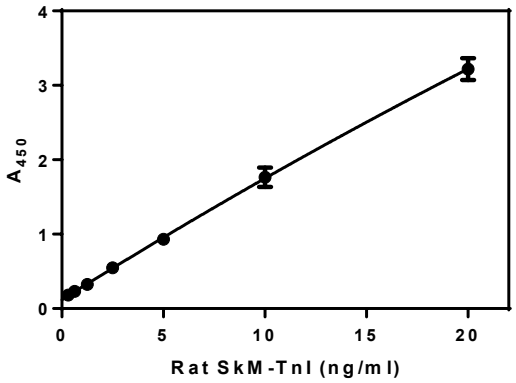
CALCULATION OF RESULTS

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 SkM-TnI (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 SkM-TnI concentration by the dilution factor to obtain the actual SkM-TnI concentration.

TYPICAL STANDARD CURVE

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

SkM-TnI (ng/ml)	Absorbance (450 nm)
20	3.218
10	1.766
5	0.933
2.5	0.549
1.25	0.325
0.625	0.231
0.313	0.180



PROCEDURAL NOTES

1. Standards should be prepared just before use.
2. HRP conjugate should be prepared shortly before use.
3. The dilution buffer supplied with the kit 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. Washing the microtiter wells prior to addition of samples is critical. It removes stabilizer that interferes with the assay.
6. 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.
7. Insufficient washing will result in poor precision and falsely elevated absorbance readings.

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For technical assistance please email us at
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[3]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.