KAMIYA BIOMEDICAL COMPANY

Rat Skeletal Muscle Troponin-I ELISA

For the quantitative determination of skeletal muscle troponin-I in rat serum or plasma.

Cat. No. KT-482

For Research Use Only.

PRODUCT INFORMATION

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PRODUCT
The ***K-ASSAY***® Rat Skeletal Muscle Troponin-I ELISA is an enzyme immunoassay for the quantitative determination of skeletal muscle troponin-I in rat serum or plasma. For research use only.

INTRODUCTION
Troponin is the contractile regulating protein complex of striated muscle. It consists of three distinct polypeptides: troponin-I, troponin-C, and troponin-T. The troponin-I subunit exists in three distinct isoforms; 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 and measurement of troponin-I in serum or plasma provides a measurement of the extent of muscle injury. This ELISA kit uses a detection antibody that is specific for the fast twitch isoform of troponin-I, thereby allowing specific evaluation of skeletal muscle injury.

PRINCIPLE
The ***K-ASSAY***® Rat Skeletal Muscle Troponin-I ELISA uses two different antibodies. A polyclonal antibody specific for skeletal muscle troponin-I is used for solid phase immobilization (on the microtiter wells). A monoclonal antibody specific for fast twitch skeletal muscle troponin-I and conjugated to horse radish peroxidase (HRP) is used for detection. The test sample is diluted and incubated in the microtiter wells for 45 minutes after which the wells are washed and HRP conjugate is added and incubated for 45 minutes. This results in troponin-I molecules being sandwiched between the immobilization and detection antibodies. The wells are then washed to remove unbound HRP-labeled antibodies and TMB Reagent (HRP substrate solution) is added and incubated for 20 minutes. If troponin-I is present a blue color develops. Color development is stopped by the addition of Stop Solution, changing the color to yellow, and optical density is measured spectrophotometrically at 450 nm. The concentration of troponin-I is proportional to the optical density of the test sample.

COMPONENTS
- Anti-SkM-TnI-coated microtiter wells, 96 wells
- Rat SkM-TnI Calibrator (lyophilized), 3 vials, reconstitute with 0.10 mL H_2O
- Calibrator Diluent, 50 mL
- Sample Diluent, 25 mL
- Anti-SkM-TnI HRP Conjugate, 11 mL
- Wash Solution (20X), 50 mL
- TMB Reagent, 11 mL
- Stop Solution (1N HCl), 11 mL

MATERIALS REQUIRED BUT NOT PROVIDED
- Pipettes: P-10, P-200 & P-1000 or equivalent
- Disposable pipette tips
- Distilled or de-ionized water
- Vortex mixer
- Absorbent paper
- Graph paper or appropriate PC graphing software
- Polypropylene microcentrifuge tubes (1.5 mL)
- Microtiter plate reader capable of reading OD at 450 nm.

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 de-ionized water.

CALIBRATOR PREPARATION
1. Equilibrate kit components to room temperature before use.

2. Reconstitute one vial of the lyophilized SkM-TnI stock by addition of 100 µL of de-ionized or distilled water. Mix gently until dissolved. The concentration of SkM-TnI in the reconstituted stock is indicated on the vial label.
3. Label 7 polypropylene tubes as 100, 50, 25, 12.5, 6.25, 3.125, 1.56 and 0 ng/mL.
4. Into the tube labeled 100 ng/mL, pipette 479.4 µL of Calibrator Diluent. Then add 20.6 µL of SkM-TnI stock and mix gently. This provides the 100 ng/mL calibrator.
5. Pipette 0.25 mL of Calibrator Diluent into the tubes labeled 50, 25, 12.5, 6.25, 3.125, 1.56 and 0 ng/mL.
6. Prepare a 50 ng/mL calibrator by diluting and mixing 0.25 mL of the 100 ng/mL calibrator with 0.25 mL of calibrator diluent in the tube labeled 50 ng/mL. Similarly prepare the 25, 12.5, 6.25, 3.125 and 1.56 ng/mL calibrators by serial dilution.

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

SAMPLE COLLECTION AND PREPARATION

Serum or plasma (EDTA) should be prepared as quickly as possible after blood collection and stored at 4°C. All samples should be similarly processed (i.e., storage times and temperatures should be the same for all samples). If samples cannot be assayed within 1-2 hours of collection they should be frozen at –70°C and thawed only once prior to use.

SAMPLE PREPARATION

In our studies, we have encountered samples with very low (≤ 4 ng/mL) and high (> 500 ng/mL) levels of troponin-I. Depending on the level of troponin-I two different methods of sample preparation are recommended.

1. Low troponin-I levels: plasma or serum samples should be diluted with 1/3rd volume of Sample Diluent (i.e., 180 µL of serum or plasma should be diluted with 60 µL of sample diluent).
2. High troponin-I levels: If samples prepared as described in 1 above give absorbance values that exceed those of the 100 ng/mL calibrator, samples pre-diluted with Sample diluent as described above should be further diluted with Calibrator diluent (i.e., one volume of sample pre-diluted as described in 1 above, should be mixed directly with one or more volumes of Calibrator diluent).
3. We recommend that samples be assayed in duplicate. Wherever possible, all samples should be similarly diluted in order to avoid minor matrix differences.

PROCEDURAL NOTES

1. Calibrators and diluted samples should be prepared immediately prior to use and should be used within 30 minutes of preparation.
2. Pipetting of conjugate, calibrators and samples into the microtiter plate should be completed within 10 minutes.

ASSAY PROCEDURE

1. Secure the desired number of coated wells in the holder.
2. Dispense 100 µL of calibrators and diluted samples into the wells (we recommend that samples be tested in duplicate).
3. Incubate on an orbital micro-plate shaker at 100-150 rpm at room temperature (18-25°C) for 45 minutes.
4. Remove the incubation mixture using either a plate washer or by flicking plate contents into an appropriate Bio-waste container.
5. Wash and empty the microtiter wells 5 times with 1x wash solution using a plate washer (400 µL/well). The entire wash procedure should be performed as quickly as possible.
6. Strike the wells sharply onto absorbent paper or paper towels to remove all residual droplets.
7. Add 100 µL of enzyme conjugate reagent into each well.
8. Incubate on an orbital micro-plate shaker at 100-150 rpm at room temperature (18-25°C) for 45 minutes.
9. Wash as detailed in 4 to 5 above.
10. Strike the wells sharply onto absorbent paper or paper towels to remove residual droplets.
11. Dispense 100 µL of TMB Reagent into each well.
12. Gently mix on an orbital micro-plate shaker at 100-150 rpm at room temperature (18-25°C) for 20 minutes.
13. Stop the reaction by adding 100 µL of Stop Solution to each well.
14. Gently mix. It is important to make sure that all the blue color changes to yellow.
15. Read the optical density at 450 nm with a microtiter plate reader *within 5 minutes*.
16. If absorbance values of samples exceed that of the 50 ng/mL calibrator, samples should be appropriately diluted and retested.

CALCULATION OF RESULTS

1. Calculate the mean absorbance value (A_{450}) for the calibrators and samples.
2. Construct a calibration curve by plotting the A_{450} values obtained for each reference calibrator against its concentration in ng/mL on graph paper, with absorbance on the vertical (y) axis and concentration on the horizontal (x) axis.

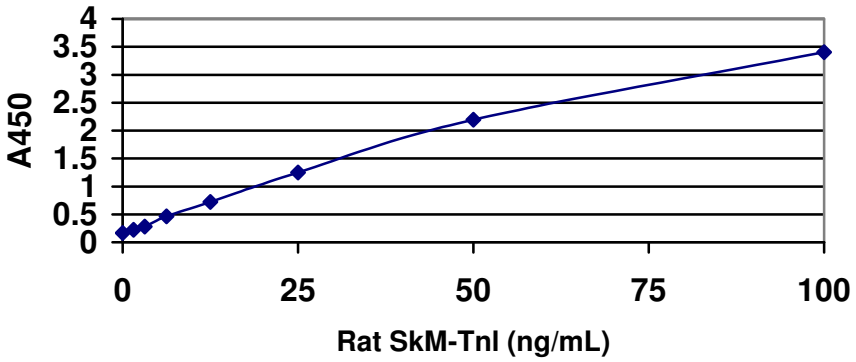
3. Using the A_{450} values for each sample, determine the corresponding concentration of SkM-TnI (ng/mL) from the calibration curve. If using graphing software, we suggest using a linear regression fit of the data.
4. Multiply the derived SkM-TnI concentrations by the dilution factor (i.e., 1.33, if the "low troponin-I level" dilution procedure was used) to obtain the actual SkM-TnI concentration.

TYPICAL CALIBRATION CURVE

Results of a typical calibration run with optical density reading at 450 nm shown on the Y axis against SkM-TnI concentration shown on the X axis are illustrated below. This calibration curve is for the illustration purpose only and should not be used to calculate unknowns. A calibration curve should be run for each assay.

SkM-TnI (ng/mL)	Absorbance (450 nm)
100	3.405
50	2.196
25	1.249
12.5	0.723
6.25	0.466
3.125	0.282
1.56	0.223
0	0.164

Typical Rat SkM-TnI Calibration Curve



STORAGE

Store the SkM-TnI stock vials at or below -20℃. Store the remainder of the kit at 4℃. Keep the microtiter plate in a sealed bag with desiccant to minimize exposure to damp air. The expiration date of the kit is indicated on the box label.

WARNINGS AND PRECAUTIONS

1. Avoid contact with 1N HCl (stop solution). It may cause skin irritation and burns. If contact occurs, wash with copious amounts of water and seek medical attention if irritation persists.
2. Do not use reagents after expiration date and do not mix or use components from kits with different lot numbers.
3. Replace caps on reagents immediately. Do not switch caps.

LIMITATIONS OF THE PROCEDURE

1. Reliable and reproducible results will be obtained when the assay procedure is carried out with a complete understanding of the package insert instructions and with adherence to good laboratory practice.

FOR RESEARCH USE ONLY

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