

RAT SKELETAL MUSCLE TROPONIN-I ELISA KIT*

RAT SKELETAL MUSCLE TROPONIN-I (SkM-TnI) ELISA

STORAGE CONDITIONS

- Store the SkM-TnI Stock vials 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 to minimize exposure to damp air

EXPIRATION

The kit expiration date (six months from the date of shipment) is indicated on the package.

BACKGROUND

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 OF THE ASSAY

The assay uses two different affinity purified antibodies. One is used for solid phase immobilization (on the microtiter wells). The second is conjugated to horse radish peroxidase (HRP) and 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.

REAGENTS AND MATERIALS PROVIDED

- Anti SkM-TnI Coated Wells (1 plate, 96 wells)
- SkM-TnI Stock (3 vials): Lyophilized rat SkM-TnI (reconstitute with 0.10 ml H₂O)
- Standard Diluent (50 ml)
- Sample Diluent (25 ml)
- Wash Buffer (20x stock, 50 ml)
- Anti SkM-TnI HRP Conjugate (11 ml)
- TMB Reagent (11 ml)
- Stop Solution (11 ml): 1N HCl

MATERIALS REQUIRED BUT NOT PROVIDED

- Distilled or deionized water
- Pipettes: P-10, P-200 & P-1000 or equivalent
- Disposable pipette tips
- Microtiter well reader capable of reading OD at 450 nm
- Vortex mixer
- Absorbent paper
- Graph paper or appropriate PC graphing software
- Polypropylene microcentrifuge tubes (1.5 ml)

WARNINGS AND PRECAUTIONS

- 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.
- Do not use reagents after expiration date and do not mix or use components from different kits.
- Replace caps on reagents immediately. Do not switch caps.

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. 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 – **USE WITHIN 30 MINUTES OF RECONSTITUTION**. The concentration of SkM-TnI in the reconstituted stock is indicated on the vial label.
3. Label 5 polypropylene tubes as 50, 25, 12.5, 6.25 and 3.125 ng/ml.
4. Into the tube labeled 50 ng/ml, pipette the volume of **Standard Diluent** detailed on the SkM-TnI stock vial label. Then add the indicated volume of SkM-TnI stock (shown on the SkM-TnI stock vial label) and mix gently. This provides the 50 ng/ml standard.
5. Pipette 0.25 ml of **Standard Diluent** into the tubes labeled 25, 12.5, 6.25 and 3.125 ng/ml
6. Prepare a 25 ng/ml standard by diluting and mixing 0.25 ml of the 50 ng/ml standard with 0.25 ml of standard diluent in the tube labeled 25 ng/ml. Similarly prepare the 12.5, 6.25 and 3.125 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 (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 studies at Life Diagnostics, Inc., 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 50 ng/ml standard, samples pre-diluted with Sample diluent as described above should be further diluted with **Standard diluent** (i.e., one volume of sample pre-diluted as described in 1 above, should be mixed directly with one or more volumes of Standard diluent).

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. Standards and diluted samples should be prepared immediately prior to use and used within 30 minutes.
2. Pipetting of all standards, samples and conjugate 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 standards 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 ($18-25^{\circ}\text{C}$) 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 ($18-25^{\circ}\text{C}$) 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 ($18-25^{\circ}\text{C}$) 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 standard, samples should be appropriately diluted and re-tested.

CALCULATION OF RESULTS

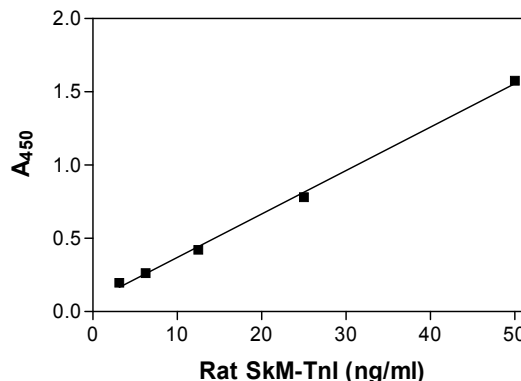
1. Calculate the mean absorbance value (A_{450}) for the standards and samples.
2. Construct a standard curve by plotting the A_{450} values obtained for each reference standard against its concentration in ng/ml on graph paper, with absorbance on the vertical (y) axis and concentration on the horizontal (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 using graphing software, we suggest using a linear regression fit of the data.
5. 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.

EXAMPLE OF STANDARD CURVE

Results of a typical standard curve with A_{450} plotted on the Y axis against cTnI 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)
50	1.576
25	0.781
12.5	0.421
6.25	0.263
3.125	0.197

Typical Rat SkM-TnI Standard Curve



LIMITATIONS OF THE PROCEDURE

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.