

# MONKEY SKELETAL MUSCLE TROPONIN-I ELISA KIT

## MONKEY 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 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 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.

### REAGENTS AND MATERIALS PROVIDED

- Anti SkM-TnI Coated Wells (1 plate, 96 wells)
- SkM-TnI Stock<sup>1</sup> (3 vials): Lyophilized SkM-TnI (reconstitute with 0.10 ml H<sub>2</sub>O)
- 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 7 polypropylene tubes as 200, 100, 50, 25, 12.5, 6.25, and 3.125 ng/ml.
4. Into the tube labeled 200 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 200 ng/ml standard.
5. Pipette 0.25 ml of **Standard Diluent** into the tubes labeled 100, 50, 25, 12.5, 6.25, and 3.125 ng/ml
6. Prepare a 100 ng/ml standard by diluting and mixing 0.25 ml of the 200 ng/ml standard with 0.25 ml of standard diluent in the tube labeled 100 ng/ml. Similarly prepare the 50, 25, 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 ( $\leq 4$  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 1/3<sup>rd</sup> volume of **Sample Diluent** (i.e., 180  $\mu$ l of serum or plasma should be diluted with 60  $\mu$ l 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 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 5 minutes.

## ASSAY PROCEDURE

1. Secure the desired number of coated wells in the holder.
2. Dispense 100  $\mu$ l 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°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 6 times with 1x wash solution using a plate washer (400  $\mu$ l/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  $\mu$ l 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  $\mu$ l 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  $\mu$ l 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. **Please Note: Due to plate reader differences, the high standard absorbance values may be out of range when read at 450 nm. If this occurs, absorbance values may be determined at 405 nm instead.**
16. If absorbance values of samples exceed that of the 200 ng/ml standard, samples should be appropriately diluted and re-tested.

## CALCULATION OF RESULTS

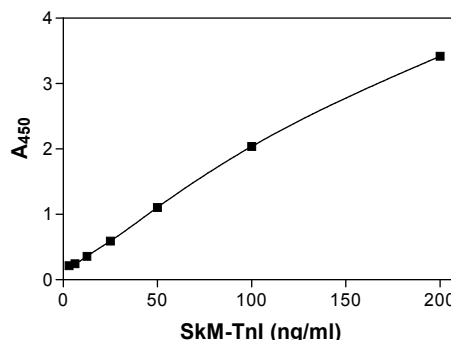
1. Calculate the mean absorbance value ( $A_{450}$ ) for the standards and samples.
2. Construct a standard curve by plotting the  $A_{450}$  values obtained for each reference standard against its concentration in ng/ml on graph paper, with absorbance on the vertical (y) axis and concentration on the 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)
200	3.418
100	2.038
50	1.106
25	0.592
12.5	0.357
6.25	0.245
3.125	0.215

**Representative Monkey 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.