HIGH SENSITIVITY RAT CARDIAC TROPONIN-I ELISA KIT
Life Diagnostics, Inc., Cat. No. CTNI-2-HS

HIGH SENSITIVITY ELISA FOR DETERMINATION OF CARDIAC TROPONIN-I IN RAT SERUM

STORAGE CONDITIONS
On receipt store the lyophilized standard at or below minus 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 label.

BACKGROUND
Troponin is the inhibitory or contractile regulating protein complex of striated muscle. It is located periodically along the thin filament of the muscle and consists of three distinct proteins: troponin I, troponin C, and troponin T. The troponin I subunit exists in three isoforms; two in fast-twitch and slow-twitch skeletal muscle fibers, and one in cardiac muscle. At the sequence level, cardiac troponin-I (cTnI) is significantly different from the skeletal isoforms and antibodies can be prepared that specifically recognize cTnI. The unique isoform and tissue specificity of cTnI are the basis for its use as a marker of cardiac muscle damage.

PRINCIPLE OF THE ASSAY
The high sensitivity cTnI ELISA recognizes an epitope on rat cTnI that is relatively resistant to proteolysis in rat serum, thereby improving detection capability. The assay uses two different affinity purified antibodies. One is used for solid phase immobilization (on the microtiter wells). The second is conjugated to horseradish peroxidase (HRP). The serum sample is allowed to react simultaneously with the two antibodies, resulting in cTnI being sandwiched between the solid phase and HRP-conjugated antibodies. After incubation for one hour at room temperature on a plate shaker the wells are washed to remove unbound HRP-conjugated antibodies. A solution of tetramethylbenzidine (TMB), an HRP substrate, is then added and incubated for 20 minutes, resulting in the development of a blue color. The color development is stopped by addition of 1N HCl, changing the color to yellow. The concentration of cTnI is proportional to the absorbance at 450 nm.

REAGENTS AND MATERIALS PROVIDED
- Anti cTnI-coated wells (1 plate, 96 wells)
- cTnI Stock: Lyophilized rat cTnI (reconstitute with 0.40 ml H_2O)
- cTnI Diluent (12 ml)
- cTnI HRP Conjugate (11 ml)
- 20x Wash Solution (50 ml)
- TMB Reagent (11 ml): HRP substrate solution
- 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
- Micro-Plate shaker/incubator with mixing speed of ~150 rpm
- Plate 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.
- Do not pipette reagents by mouth.

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
Sufficient reagents are provided for the preparation of at least two standard curves.

1. Equilibrate kit components to room temperature before use.
2. Reconstitute the lyophilized cTnI stock by addition of 400 μl of deionized or distilled water. Mix gently several times over a period of 5-10 minutes. The concentration of cTnI in the reconstituted stock is indicated on the vial label.
3. Label 7 polypropylene tubes as 10, 5, 2.5, 1.25, 0.625, 0.312, and 0.156 ng/ml.
4. Into the tube labeled 10 ng/ml, pipette the volume of cTnI diluent detailed on the cTnI stock vial label. Then add the indicated volume of cTnI stock (shown on the cTnI stock vial label) and mix gently. This provides the 10 ng/ml standard.
5. Pipette 0.25 ml of cTnI diluent into the tubes labeled 5, 2.5, 1.25, 0.625, 0.312 and 0.156 ng/ml.
6. 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 as 5 ng/ml. Similarly prepare the 2.5, 1.25, 0.625, 0.312 and 0.156 ng/ml standards by serial dilution.

NOTE: The reconstituted cTnI stock should be frozen immediately after use. It remains stable in frozen form for at least 1 month at -20°C and 6 months at -70°C. Discard the working 10 - 0.156 ng/ml standards after use.

SAMPLE COLLECTION AND PREPARATION

Serum 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). If serum samples cannot be assayed immediately, they should be frozen at –70°C and thawed only once prior to use.

ASSAY PROCEDURE

1. Secure the desired number of coated wells in the holder.
2. **Dispense 100 µl of cTnI HRP Conjugate into each well.**
3. Dispense 100 µl of standards and samples into appropriate wells.
4. Thoroughly mix for 10-15 seconds. It is very important to mix completely.
5. Incubate on an orbital shaker (150 rpm) at room temperature (18-25°C) for 60 minutes.
6. Remove the incubation mixture using a plate washer or by flicking the plate contents into a bio-waste container.
7. Wash and empty the microtiter wells 5 times with 1x wash solution. This may be performed using either a plate washer (400 µl/well) or a squirt bottle. The entire wash procedure should be performed as quickly as possible.
8. Strike the wells sharply onto absorbent paper or paper towels to remove all residual droplets.
9. Dispense 100 µl of TMB Reagent into each well. Gently mix for 5 seconds.
10. Incubate on an orbital shaker (150 rpm) at room temperature for 20 minutes.
11. Stop the reaction by adding 100 µl of Stop Solution to each well.
12. Gently mix until all the blue color changes to yellow.
13. Read absorbance at 450 nm with a plate reader within 15 minutes. ***Please Note: Due to plate reader differences, the high standard absorbance values may be out of range occasionally. If this occurs, absorbance values may be determined at 405 nm instead.***
14. If absorbance values exceed the high standard, the samples should be appropriately diluted with sample diluent (catalog number 2010-HSD, Life Diagnostics, Inc.) and re-determined. Samples with absorbance values below those of the 0.156 ng/ml standard should be assigned a zero troponin-I value.

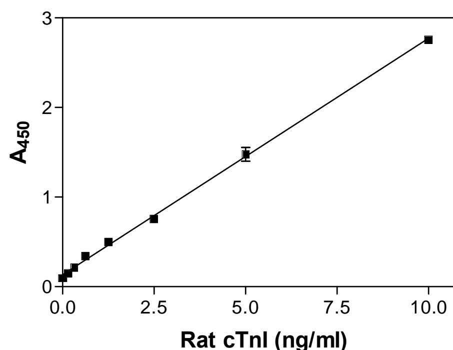
CALCULATION OF RESULTS

1. Calculate the mean absorbance value (A_{450}) for each of the standards and samples.
2. Construct a standard curve by plotting the A_{450} value 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 cTnI (ng/ml) from the standard curve.
4. If available, graphing software may be used to analyze the data. Depending on the range of the standard curve used, we find that good fits of the data may be obtained with linear regression analysis or using a two-site binding model. Alternatively, standard curves may be generated using a point-to-point fit.

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.

cTnI (ng/ml)	Absorbance (450 nm)
10	2.760
5	1.478
2.5	0.760
1.25	0.501
0.625	0.340
0.313	0.212
0.156	0.150



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 instructions and with adherence to good laboratory practice.
2. Plasma cannot be used with this kit.

REFERENCES

1. Wang P and Chatham JC. Onset of diabetes in Zucker diabetic fatty (ZDF) rats leads to improved recovery of function after ischemia in the isolated perfused heart. Am J Physiol Endocrinol Metab. 286:E725-E736 (2004)
2. Liu J, Pang Y, Chang T, Bounelis P, Chatham JC, and Marchase RB. Increased hexoseamine biosynthesis and protein O-GlcNAc levels associated with myocardial protection against calcium paradox and ischemia. J Mol. Cell Cardiol. 40 303-312 (2006)

Rev 040915NC

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