KAMIYA BIOMEDICAL COMPANY

Rat Cardiac Troponin-I ELISA

For the quantitative determination of cardiac troponin-I in rat serum.

Cat. No. KT-478

For Research Use Only.

PRODUCT INFORMATION

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PRODUCT
The ***K-ASSAY***® Rat Cardiac Troponin-I ELISA is an enzyme immunoassay for the quantitative determination of cardiac troponin-I in rat serum. For research use only.

INTRODUCTION
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
The ***K-ASSAY***® Rat Cardiac Troponin-I 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 one hour incubation at room temperature on a plate shaker, the wells are washed to remove unbound HRP-conjugated antibodies. A solution of TMB (Tetramethylbenzidine), an HRP substrate, is then added and incubated for 20 minutes, resulting in the development of a blue color. The color development is stopped with the addition of 1N HCl changing the color to yellow. The concentration of cTnI is proportional to the absorbance at 450 nm.

COMPONENTS
- Anti-cTnI-coated microtiter wells, 96 wells
- Rat cTnI Calibrator (lyophilized), reconstitute with 0.40 mL H_2O
- cTnI Diluent, 12 mL
- cTnI 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

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

1. Equilibrate kit components to room temperature before use.
2. Reconstitute the lyophilized cTnI stock by addition of 400 µL of de-ionized 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 432.8 µL of cTnI diluent. Then add 67.2 µL of cTnI calibrator and mix gently. This provides the 10 ng/mL calibrator.
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 calibrator by diluting and mixing 0.25 mL of the 10 ng/mL calibrator 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 calibrators by serial dilution.

Note: The reconstituted cTnI calibrator 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 calibrators 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.

PROCEDURAL NOTES

1. Calibrators 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.
3. We recommend that calibrators and samples be run in duplicate.
4. It is recommended that the wells be read within 5 minutes following addition of Stop Solution.

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 calibrators and diluted samples into the appropriate wells.
4. Thoroughly mix for 10-15 seconds. It is very important to mix completely.
5. Incubate at room temperature (18-25°C) on a plate shaker (150 rpm) for one hour.
6. Remove the incubation mixture using a plate washer or by flicking 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 with a squirt bottle. The entire wash procedure should be performed as quickly as possible.
8. Strike the wells sharply onto adsorbent paper or paper towels to remove all residual droplets.
9. Dispense 100 µL of TMB Reagent solution into each well. Gently mix for 5 seconds.
10. Incubate on a plate 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. It is important to make sure that all the blue color changes to yellow.
13. Read absorbance at 450 nm with a microtiter plate reader *within 15 minutes.* **Please note: Due to plate reader differences, the high calibrator 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 calibrator, the samples should be appropriately diluted with sample diluent and re-determined. Samples with absorbance values below those of the lowest calibrator should be assigned a zero troponin-I value.

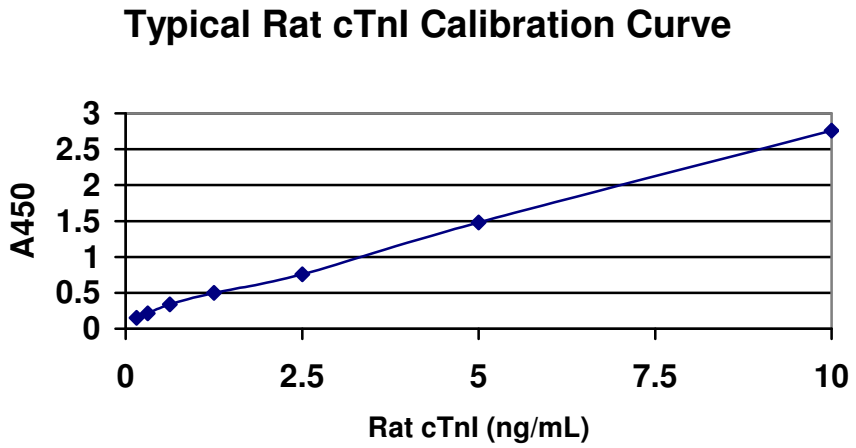
CALCULATION OF RESULTS

1. Calculate the mean absorbance values (A_{450}) for each set of reference calibrators and samples.
2. Construct a calibration curve by plotting the mean absorbance obtained from each reference calibrator against its concentration in ng/mL on graph paper, with absorbance values on the vertical or Y-axis and concentration on the horizontal or X-axis.
3. Using the mean absorbance value for each sample, determine the corresponding concentration of cTnI in ng/mL from the calibration curve.
4. If available, graphing software may be used to analyze the data. Depending on the range of the calibration 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, calibration curves may be generated using a point-to-point fit.

TYPICAL CALIBRATION CURVE

Results of a typical calibration run with optical density reading at 450 nm shown on the Y axis against cTnI 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.

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



STORAGE

The lyophilized reference calibrator should be stored at -20℃ for optimum stability. The remainder of the kit should be stored refrigerated 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. Do not pipette reagents by mouth.
4. 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.
2. Plasma cannot be used with this kit.

FOR RESEARCH USE ONLY

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