

KAMIYA BIOMEDICAL COMPANY

Rat Cardiac Troponin-I, High Sensitive ELISA

For the high sensitive quantitative determination of cardiac troponin-I in rat serum or plasma.

Cat. No. KT-639

For Research Use Only.

PRODUCT INFORMATION

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PRODUCT

The **K-ASSAY®** Rat Cardiac Troponin-I, High Sensitive ELISA is an enzyme immunoassay for the high sensitive quantitative determination of cardiac troponin-I in rat serum or plasma. For research use only.

STORAGE

When the kit is received, store the lyophilized calibrator at or below -20°C. Store the remainder of the kit in the refrigerator at 4°C. Keep the microtiter plate in a sealed bag with desiccant. The kit expiration date is indicated on the box label.

INTRODUCTION

Troponin is the contractile regulating protein complex of striated muscle. It consists of three subunits: troponin I, T, and C. Troponin-I exists in three isoforms; one in fast-twitch skeletal muscle, one in slow-twitch skeletal muscle, and one in cardiac muscle. After muscle injury by trauma or ischemia, troponin is released into the bloodstream and its levels correlate well with tissue injury. The sequence of cardiac troponin-I (cTnI) is significantly different from the skeletal muscle isoforms, thus allowing generation of specific anti-cTnI antibodies and development of cTnI specific immunoassays. Because cTnI is uniquely expressed in the heart it provides a specific biomarker of cardiac damage.

PRINCIPLE

The **K-ASSAY®** Rat Cardiac Troponin-I, High Sensitive ELISA uses two affinity purified antibodies. A rabbit anti-cTnI polyclonal antibody is used for solid phase immobilization (on the microtiter wells). A goat anti-cTnI peptide-specific polyclonal antibody is conjugated to horseradish peroxidase (HRP) and used for detection. Samples (serum or plasma) and calibrators (200 µL) are pipetted into the microtiter wells and incubated for 2 hours on a plate shaker. After washing the wells, 100 µL of diluent and 100 µL of HRP-conjugated anti-cTnI are pipetted into each of the microtiter wells. The plate is incubated for one hour on a plate shaker. During this step, cTnI becomes sandwiched between the solid phase and HRP-conjugated antibodies. The wells are then washed to remove unbound HRP-conjugated antibodies. TMB, an HRP substrate (100 µL), is then added and incubated for 20 minutes, resulting in the development of a blue color. The color development is stopped by the addition of 1N HCl (100 µL), changing the color to yellow, and absorbance at 450 nm is measured. The concentration of cTnI is proportional to the absorbance at 450 nm and is derived from a calibration curve.

COMPONENTS

- Anti-cTnI-coated microtiter plate (96 wells 12 x 8-well strips)
- cTnI Stock: Lyophilized rat cTnI
- cTnI Diluent (25 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 de-ionized water
- Pipettes & tips: P-10, P-200 & P-1000 or equivalent
- Plate shaker
- Plate washer
- 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

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 different kits.
3. 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 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 adding 200 μ L of de-ionized or distilled water. Mix gently several times over a period of 5 minutes.
3. Label 7 polypropylene tubes as 2.5, 1.25, 0.625, 0.312, 0.156, 0.078 and 0.039 ng/mL.
4. Into the tube labeled 2.5 ng/mL, pipette 970.86 μ L of cTnI diluent. Then add 29.14 μ L of cTnI stock and mix gently. This provides the 2.5 ng/mL calibrator.
5. Pipette 0.50 mL of cTnI diluent into the tubes labeled 1.25, 0.625, 0.312, 0.156, 0.078 and 0.039 ng/mL.
6. Prepare a 1.25 ng/mL calibrator by diluting and mixing 0.50 mL of the 2.5 ng/mL calibrator with 0.50 mL of diluent in the tube labeled as 1.25 ng/mL. Similarly prepare the 0.625, 0.312, 0.156, 0.078, and 0.039 ng/mL calibrators 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 calibrators after use.

SAMPLE COLLECTION AND PREPARATION

Serum or plasma 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 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 200 μ L of calibrators and samples into appropriate wells.
3. Incubate on an orbital shaker (150 rpm) at 25°C for 2 hours.
4. Using a plate washer, wash the microtiter wells 5 times with 1X wash solution (400 μ L per well). The entire wash procedure should be performed as quickly as possible.
5. Strike the wells sharply onto adsorbent paper or paper towels to remove all residual droplets.
6. Add 100 μ L of cTnI diluent to each well.
7. Add 100 μ L of HRP conjugate to each well.
8. Incubate on an orbital shaker (150 rpm) at 25°C for 1 hour.
9. Wash the plate as described in steps 4 and 5.
10. Dispense 100 μ L of TMB Reagent into each well.
11. Incubate on an orbital shaker (150 rpm) at 25°C for 20 minutes.
12. Stop the reaction by adding 100 μ L of Stop Solution to each well.
13. Gently mix until all the blue color changes to yellow.
14. Read absorbance at 450 nm with a plate reader within 5 minutes.
15. If absorbance values exceed the high calibrator, the samples should be further diluted with cTnI diluent and re-determined.

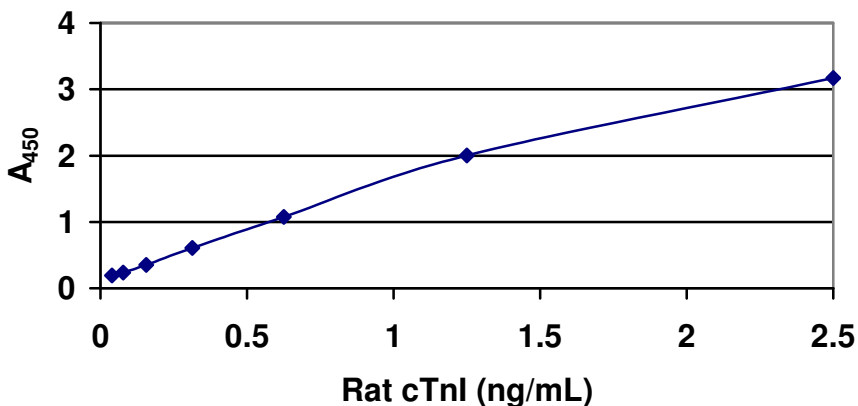
CALCULATION OF RESULTS

1. Calculate the mean absorbance values (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 cTnI (ng/mL) from the calibration curve.
4. If available, graphing software should be used to analyze the data. Depending on the range of the calibration curve used for analysis, good fits may be obtained with linear regression analysis or by fitting the data to a polynomial second order equation. We recommend the latter option.

REPRESENTATIVE CALIBRATION CURVE

Results of a typical calibration curve with A_{450} plotted on the Y axis against cTnl concentrations on the X axis are shown below. **NOTE:** This calibration curve is for the purpose of illustration only.

cTnl (ng/mL)	Absorbance (450 nm)
2.5	3.169
1.25	2.001
0.625	1.075
0.313	0.606
0.156	0.350
0.078	0.235
0.039	0.194



PROCEDURAL NOTES

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. Calibrators should be used within 30 minutes of preparation.
3. Pipetting of all reagents into the microtiter plate should be completed within 5 minutes at each step (we strongly recommend the use of multipipetors).
4. Calibrators and samples should be run in duplicate.

FOR RESEARCH USE ONLY

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