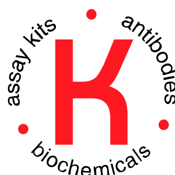



KAMIYA BIOMEDICAL COMPANY

# 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

## Rat Cardiac Troponin-I, High Sensitive ELISA

### Cat. No. KT-639

## 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
Store the lyophilized stock at or below -20°C. The remainder of the kit should be stored at 4°C and the microtiter plate should be kept in a sealed bag with desiccant. Kits will remain stable until the expiration date.

## INTRODUCTION
Cardiac troponin-I (CTNI) is a component of the troponin complex that regulates muscle contraction. After cardiac injury, CTNI is released into the blood. Because it is expressed specifically in the heart it is an excellent biomarker of cardiac injury. In humans, CTNI levels peak 12-24 hours after injury, returning to baseline within 2-6 days. In mice, levels peak as early as 1 hour and return to normal within 1-3 days.

## PRINCIPLE
The ELISA uses two different antibodies that recognize a relatively protease-resistant epitope on CTNI. One is used for solid phase immobilization (microtiter wells). The second is conjugated to horse radish 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-conjugate is pipetted into the wells. The plate is incubated for one hour. During this step, CTNI, if present, is sandwiched between the immobilization and HRP-conjugated antibodies. The wells are then washed to remove unbound HRP-conjugate. TMB is added and incubated for 20 minutes. If CTNI is present a blue color develops. Color development is stopped by addition of Stop solution, changing the color to yellow. Absorbance is measured at 450 nm. The concentration of CTNI is proportional to absorbance and is derived from a calibration curve.

## COMPONENTS
- Anti-CTNI coated plate (12 x 8-well strips)
- CTNI Calibrator Stock, lyophilized
- Diluent: 25 mL
- HRP Conjugate: 11 mL
- 20X Wash solution: 50 mL
- TMB: 11 mL
- Stop solution: 11 mL

## MATERIALS REQUIRED BUT NOT PROVIDED
- Pipettors and tips
- Distilled or deionized water
- Polypropylene or glass tubes
- Vortex mixer
- Absorbent paper or paper towels
- Plate incubator/shaker
- Plate washer
- Plate reader capable of measuring absorbance at 450 nm
- Curve fitting software

## GENERAL INSTRUCTIONS
1. All reagents should be allowed to reach room temperature before use.

2. Reliable and reproducible results will be obtained when the assay is carried out with a complete understanding of the instructions and with adherence to good laboratory practice.
3. The wash procedure is critical. Insufficient washing will result in poor precision and falsely elevated absorbance readings.
4. Laboratory temperature will influence absorbance readings. Our ELISA kits are calibrated using shaking incubators set at 150 rpm and 25°C. Performance of the assay at lower temperatures will result in lower absorbance values.

## 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

1. Reconstitute the lyophilized CTNI stock by adding 200 µL of de-ionized or distilled water. Mix gently until dissolved.
2. Label 7 polypropylene tubes as 2.5, 1.25, 0.625, 0.313, 0.156, 0.078 and 0.039 ng/mL.
3. Into the tube labeled 2.5 ng/mL, pipette 970.86 µL of CTNI diluent. Then add 29.14 µL of CTNI stock and mix gently. This provides the 2.5 ng/mL calibrator.
4. Pipette 250 µL of diluent into the tubes labeled 1.25, 0.625, 0.313, 0.156, 0.078 and 0.039 ng/mL.
5. Prepare a 1.25 ng/mL calibrator by diluting and mixing 250 µL of the 2.5 ng/mL calibrator with 250 µL of diluent in the tube labeled 1.25 ng/mL. Similarly prepare the remaining calibrators by two-fold serial dilution.

**The reconstituted CTNI stock should be frozen immediately after use. It remains stable when frozen 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. Undiluted serum can be used with this kit. If dilution is necessary, use diluent included with kit. Other diluents must not be used.

## ASSAY PROCEDURE

1. Secure the desired number of 8-well strips in the holder. Unused strips should be stored in the re-sealed bag with desiccant at 4°C for future use.
2. Dispense 200 µL of calibrators and samples into the wells (we recommend that calibrators and samples be run in duplicate). 100 µL of sample may be used if volume is limiting. However, the volume of all other samples and calibrators used in the assay must also be 100 µL. Absorbance values will be slightly lower.
3. Incubate on a plate shaker at 150 rpm and 25°C for 2 hours.
4. Empty and wash the microtiter wells 5x with 1X wash solution using a plate washer (400 µL/well).
5. Strike the wells sharply onto adsorbent paper or paper towels to remove all residual droplets.
6. Add 100 µL of diluent to each well.
7. Add 100 µL of HRP-conjugate to each well.
8. Incubate on a plate shaker at 150 rpm and 25°C for one hour.
9. Empty and wash the microtiter wells 5x with 1X wash solution using a plate washer (400 µL/well).
10. Strike the wells sharply onto adsorbent paper or paper towels to remove all residual droplets.
11. Dispense 100 µL of TMB into each well.
12. Incubate on a plate shaker at 150 rpm and 25°C for 20 minutes.
13. After 20 minutes, 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 absorbance at 450 nm with a plate reader within 5 minutes.

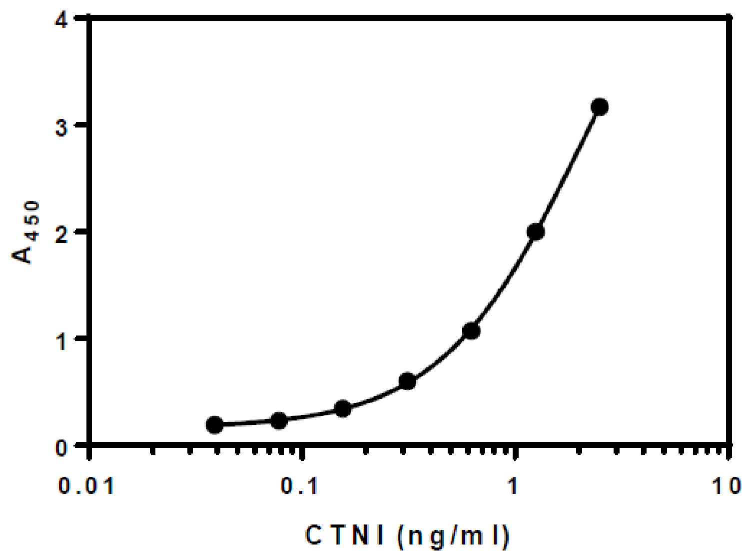
## CALCULATION OF RESULTS

1. Using curve fitting software, construct a calibration curve by plotting absorbance values of the calibrators versus $\log_{10}$ of the concentration.
2. Fit the calibration curve to a four-parameter logistic regression (4PL) equation (x axis = $\log_{10}$ concentration) and determine the concentration of the samples.
3. Multiply the derived concentration by the dilution factor (if applicable) to determine the actual concentration in the original sample.
4. If the $A_{450}$ values fall outside the calibration curve, samples should be diluted appropriately and re-tested.

## TYPICAL CALIBRATION CURVE

A typical calibration curve is shown on the next page. This is for illustration only. A calibration curve must be generated for each experiment.

| CTNI (ng/mL) | $A_{450}$ |
|---|---|
| 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 |




**FOR RESEARCH USE ONLY**

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