

KAMIYA BIOMEDICAL COMPANY

Rat Cardiac Troponin-I ELISA

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

Cat. No. KT-480

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 plasma. For research use only.

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

This assay is intended for use with plasma samples. 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. Plasma samples are first diluted with three volumes of plasma diluent. Calibrators and diluted samples are then incubated in the microtiter wells with HRP conjugate for one hour. This results in CTNI molecules being sandwiched between the immobilization and detection antibodies. The wells are 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, and 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 Stock Calibrator
- Calibrator diluent, 25 mL
- Plasma 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

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.

GENERAL INSTRUCTIONS

1. All reagents should be allowed to reach room temperature before use.
2. After thawing, a precipitate may be apparent in the calibrator diluent. The precipitate should be removed by centrifugation for 5 min at ~3,000 rpm in a bench-top centrifuge. Use the clear supernatant.
3. 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.

- The wash procedure is critical. Insufficient washing will result in poor precision and falsely elevated absorbance readings.
- 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

- Reconstitute the lyophilized CTNI stock with 200 μ L of de-ionized or distilled water. Mix gently until dissolved.
- Label 7 polypropylene tubes as 10, 5, 2.5, 1.25, 0.625, 0.313 and 0.156 ng/mL.
- Into the tube labeled 10 ng/mL, pipette 443.1 μ L of calibrator diluent. Then add 56.9 μ L of calibrator and mix gently. This provides the 10 ng/mL calibrator.
- Pipette 0.25 mL of calibrator diluent into the tubes labeled 5, 2.5, 1.25, 0.625, 0.313 and 0.156 ng/mL.
- 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 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

Plasma (EDTA, citrate or heparin) 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 plasma samples cannot be assayed within 4 hours of collection they should be frozen at -70°C and thawed only once prior to use. We recommend that samples be assayed in duplicate. Prior to assay, plasma samples should be diluted four-fold with plasma diluent. This can easily be accomplished by mixing 100 μ L of each plasma sample with 300 μ L of plasma diluent in a polypropylene micro centrifuge tube.

ASSAY PROCEDURE

- 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.
- Add 100 μ L of HRP-conjugate into each well.
- Dispense 100 μ L of calibrators and diluted samples into the wells (we recommend that calibrators and samples be run in duplicate).
- Incubate on a plate shaker at 150 rpm and 25°C for one hour.
- Empty and wash the microtiter wells 5x with 1x wash solution using a plate washer (400 μ L/well).
- Strike the wells sharply onto absorbent paper or paper towels to remove all residual droplets.
- Dispense 100 μ L of TMB into each well.
- Incubate on an orbital micro-plate shaker at 150 rpm and 25°C for 20 minutes.
- After 20-minutes, stop the reaction by adding 100 μ L of Stop solution to each well.
- Gently mix. It is important to make sure that all the blue color changes to yellow.
- Read absorbance at 450 nm with a plate reader within 5 minutes.

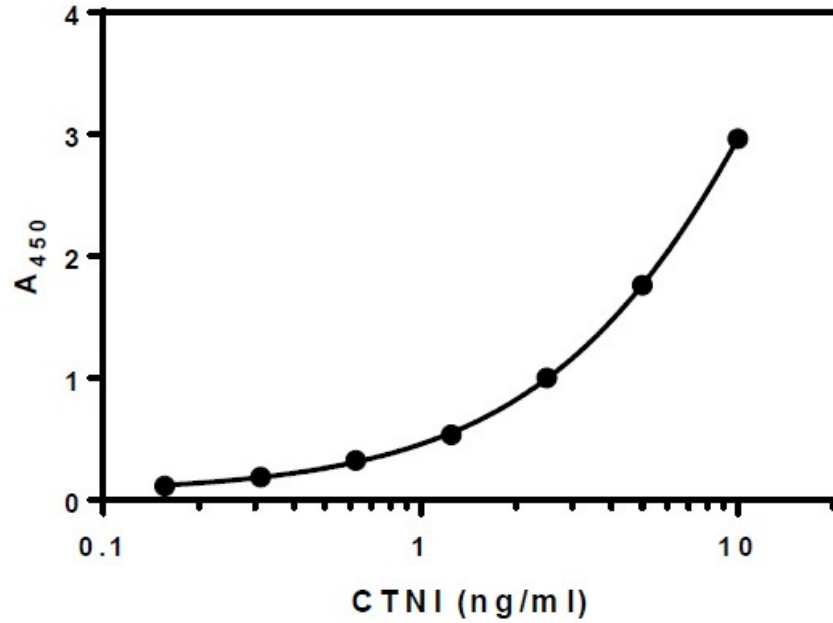
CALCULATION OF RESULTS

- Using curve fitting software, construct a calibration curve by plotting absorbance values of the calibrators versus \log_{10} of the concentration.
- Fit the calibration curve to a four-parameter logistic regression (4PL) equation (x axis = \log_{10} concentration) and determine the concentration of the samples (derive the antilog).
- Multiply the derived concentration by the dilution factor to determine the actual concentration in the original sample.
- If the A_{450} values fall outside the calibration curve, samples should be diluted appropriately and re-tested. If further dilution is required, freshly thawed plasma samples should first be diluted with three volumes of plasma diluent (4-fold dilution). The resulting mixture should then be diluted with calibrator diluent.

TYPICAL CALIBRATION CURVE

A typical calibration curve is shown below. This is for illustration only. A calibration curve must be generated for each experiment.

CTNI (ng/mL)	A450
10	2.965
5	1.766
2.5	1.004
1.25	0.539
0.625	0.329
0.313	0.193
0.156	0.119



FOR RESEARCH USE ONLY

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