KAMIYA BIOMEDICAL COMPANY

Mouse Cardiac Troponin-I ELISA

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

Cat. No. KT-469

For Research Use Only.

PRODUCT INFORMATION

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PRODUCT
The ***K-ASSAY®*** Mouse Cardiac Troponin-I ELISA is an enzyme immunoassay for the quantitative determination of cardiac troponin-I in mouse serum. 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
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. Calibrators and serum samples are 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 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
- Diluent, 12 mL
- HRP Conjugate, 11 mL
- Wash Solution (20X), 50 mL
- TMB, 11 mL
- Stop Solution, 11 mL

MATERIALS REQUIRED BUT NOT PROVIDED
- Pipettors and tips
- Distilled or de-ionized 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

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 calibrator with de-ionized or distilled water as detailed on the vial label. Mix gently until dissolved.
2. Label 7 polypropylene tubes as 10, 5, 2.5, 1.25, 0.625, 0.312, and 0.156 ng/mL.
3. Into the tube labeled 10 ng/mL, pipette 437.1 µL of diluent. Then add 62.9 µL of calibrator and mix gently. This provides the 10 ng/mL calibrator.
4. Pipette 250 µL of diluent into the tubes labeled 5, 2.5, 1.25, 0.625, 0.312 and 0.156 ng/mL.

5. Prepare a 5 ng/mL calibrator by diluting and mixing 250 µL of the 10 ng/mL calibrator with 250 µL of diluent in the tube labeled 5 ng/mL. Similarly prepare the remaining calibrators by two-fold serial dilution.

The reconstituted CTNI calibrator should be frozen immediately after use. It remains stable when frozen for at least 1 month at -20℃ and 6 months at -70℃. Discard the working calibrators after use.

SAMPLE COLLECTION AND PREPARATION

Serum should be prepared as quickly as possible after blood collection and stored at 4℃. 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℃ and thawed only once prior to use. Undiluted serum can be used with this kit. If dilution is necessary, use diluent provided. Other diluents must not be used. Plasma cannot be used with this kit.

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℃. Performance of the assay at lower temperatures will result in lower absorbance values.

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℃ for future use.
2. Dispense 100 µL of calibrators and samples into the wells (we recommend that calibrators and samples be run in duplicate).
3. Add 100 µL of HRP-conjugate into each well.
4. Incubate on a plate shaker at 150 rpm and 25℃ for one hour.
5. Empty and wash the microtiter wells 5x with 1X wash solution using a plate washer (400 µL/well).
6. Strike the wells sharply onto adsorbent paper or paper towels to remove all residual droplets.
7. Dispense 100 µL of TMB into each well.
8. Incubate on a plate shaker at 150 rpm and 25℃ for 20 minutes.
9. After 20 minutes, stop the reaction by adding 100 µL of Stop solution to each well.
10. Gently mix. It is important to make sure that all the blue color changes to yellow.
11. Read absorbance at 450 nm with a plate reader within 5 minutes.

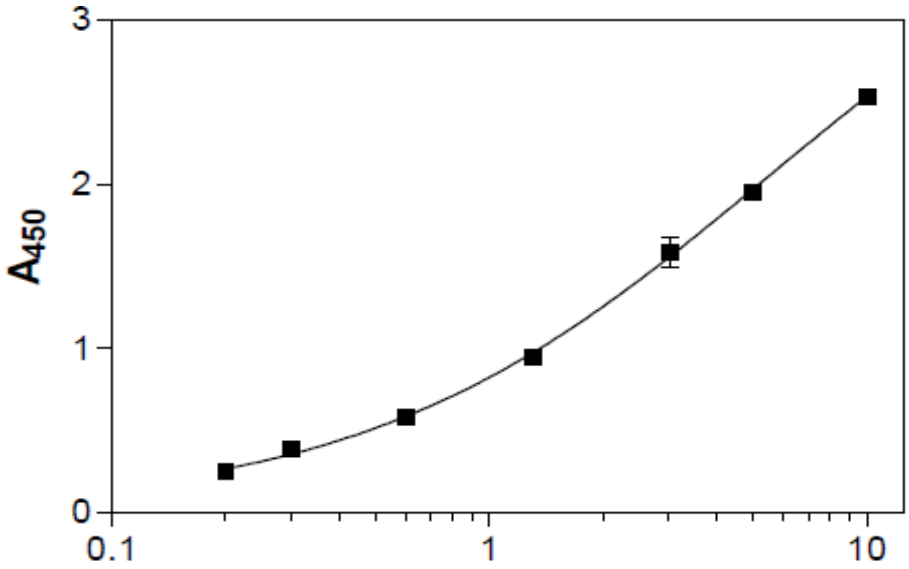
CALCULATION OF RESULTS

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

CTNI (ng/mL)	A_{450}
10	2.538
5	1.951
2.5	1.463
1.25	0.964
0.625	0.623
0.313	0.400
0.156	0.247



STORAGE

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

FOR RESEARCH USE ONLY

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