KAMIYA BIOMEDICAL COMPANY

Rat & Mouse Cardiac Myosin Light Chain-1 ELISA

For the quantitative determination of cardiac myosin light chain-1 in rat or mouse serum or plasma.

Cat. No. KT-483

For Research Use Only.

PRODUCT INFORMATION

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PRODUCT
The ***K-ASSAY®*** Rat & Mouse Cardiac Myosin Light Chain-1 ELISA is an enzyme immunoassay for the quantitative determination of cardiac myosin light chain-1 in rat or mouse serum or plasma. For research use only.

INTRODUCTION
Myosin light chains are released into the circulation following muscle injury and provide useful biomarkers of muscle damage. Myosin light chain-1 (MLC-1) is expressed as different but immunologically related isoforms in cardiac and skeletal muscle. The antibodies used in this ELISA kit are approximately ten times more sensitive toward cardiac MLC-1 (CMLC-1). The assay provides a useful tool for assessment of cardiac injury in the absence of skeletal muscle injury.

PRINCIPLE
The assay uses two CMLC-1 monoclonal antibodies. One is used for solid phase immobilization (microtiter wells). The other is conjugated to horseradish peroxidase (HRP) and used for detection. Calibrators and diluted samples are incubated in the microtiter wells for one hour. The wells are subsequently washed. HRP conjugate is added and incubated for one hour. This results in CMLC-1 molecules being sandwiched between the immobilization and detection antibodies. The wells are then washed to remove unbound HRP-conjugate and TMB is added and incubated for 20 minutes. If CMLC-1 is present a blue color develops. Color development is stopped by the addition of Stop solution, changing the color to yellow, and absorbance is measured at 450 nm. The concentration of CMLC-1 is proportional to absorbance and is derived from a calibration curve.

COMPONENTS
- CMLC-1 antibody coated plate (12 x 8-well strips)
- CMLC-1 calibrator
- 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 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 calibrator with the volume of distilled or de-ionized water on the vial label. Mix gently until dissolved.
2. Label 6 microcentrifuge tubes as 2.5, 1.25, 0.625, 0.312, 0.156 and 0.078 ng/mL.
3. Into the tube labeled 2.5 ng/mL, pipette 0.494 mL of diluent. Then add 5.42 µL of calibrator and mix. This provides the 2.5 ng/mL calibrator.

4. Dispense 250 µL of diluent into the tubes labeled 1.25, 0.625, 0.312, 0.156, and 0.078 ng/mL.
5. Pipette 250 µL of the 2.5 ng/mL calibrator into the tube labeled 1.25 ng/mL and mix. This provides the 1.25 ng/mL calibrator.
6. Similarly prepare the remaining calibrators by two-fold serial dilution.

Please Note: The unused reconstituted calibrator should be aliquoted and stored frozen at or below -20°C (within 1 hour of reconstitution) if future use is intended.

SAMPLE PREPARATION

Plasma and 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 for all samples). If samples cannot be assayed within 4 hours of collection they should be frozen at –70°C and thawed only once. We recommend that samples be assayed in duplicate. Optimum dilution should be determined by the end user. Samples should only be diluted with the diluent supplied with the 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 procedure 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.

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 100 µL of calibrators and samples into the wells (we recommend that calibrators and samples be run in duplicate).
3. Incubate on an orbital micro-plate shaker at 150 rpm and 25°C for one hour.
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 absorbent paper or paper towels to remove all residual droplets.
6. Add 100 µL of HRP-conjugate into each well.
7. Incubate on a plate shaker at 150 rpm and 25°C for one hour.
8. Wash as detailed above.
9. Strike the wells sharply onto absorbent paper or paper towels to remove residual droplets.
10. Dispense 100 µL of TMB into each well.
11. Incubate on an orbital micro-plate shaker at 150 rpm and 25°C for 20 minutes.
12. After 20 minutes, stop the reaction by adding 100 µL of Stop solution to each well.
13. Gently mix. It is important to make sure that all the blue color changes to yellow.
14. Read absorbance at 450 nm with a plate reader within 5 minutes.

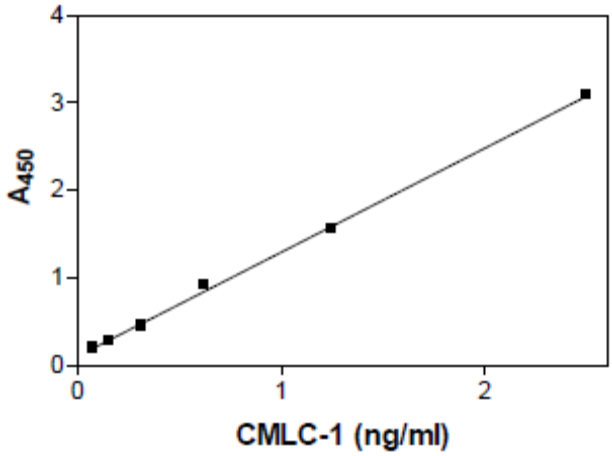
CALCULATION OF RESULTS

1. Using curve fitting software, construct a calibration curve by plotting absorbance values of the calibrators versus concentration.
2. Fit the calibration curve to an appropriate model and derive the concentration of the samples (we recommend using a single site, total and nonspecific binding model).
3. Multiply the derived concentration by the dilution factor to obtain the actual concentration in the sample.
4. If the A_{450} values of samples fall outside the calibration curve, samples should be diluted appropriately and re-tested.

TYPICAL CALIBRATION CURVE

A typical calibration curve is shown below. This curve is for illustration only and should not be used to calculate unknowns. Each user should obtain his or her data and calibration curve in each experiment.

CMLC-1 (ng/mL)	A_{450}
2.5	3.081
1.25	1.553
0.625	0.905
0.313	0.448
0.156	0.283
0.078	0.197



STORAGE

The lyophilized calibrator must be stored at or below -20℃ when received. The remainder of the kit should be stored at 4℃. The microtiter plate should be kept in a sealed bag with desiccant. Kits will remain stable until the expiration date shown on the labels if stored as described.

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3. Into the tube labeled 2.5 ng/mL, pipette 0.494 mL of diluent. Then add 5.42 µL of calibrator and mix. This provides the 2.5 ng/mL calibrator.

4. Dispense 250 µL of diluent into the tubes labeled 1.25, 0.625, 0.312, 0.156, and 0.078 ng/mL.
5. Pipette 250 µL of the 2.5 ng/mL calibrator into the tube labeled 1.25 ng/mL and mix. This provides the 1.25 ng/mL calibrator.
6. Similarly prepare the remaining calibrators by two-fold serial dilution.

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10. Dispense 100 µL of TMB into each well.
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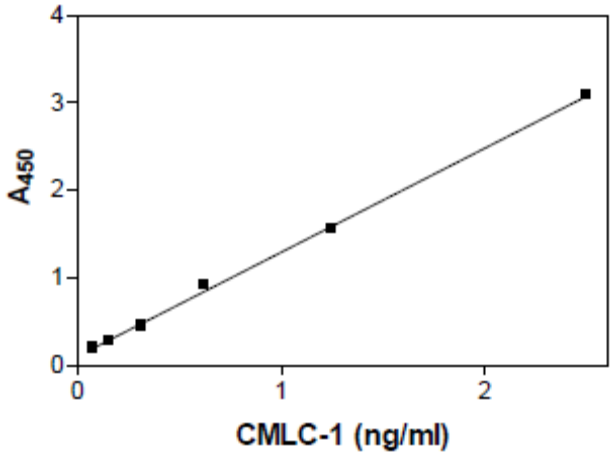

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