

KAMIYA BIOMEDICAL COMPANY

Rat & Mouse Skeletal Muscle Myosin Light Chain-1 ELISA

**For the quantitative determination of skeletal muscle myosin light chain-1 in rat
and mouse serum and plasma.**

Cat. No. KT-485

For Research Use Only.

PRODUCT INFORMATION

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PRODUCT

The **K-ASSAY®** Rat & Mouse Skeletal Muscle Myosin Light Chain-1 ELISA is an enzyme immunoassay for the quantitative determination of skeletal muscle myosin light chain-1 in rat and mouse serum and 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 skeletal muscle and heart. The antibodies used in this ELISA kit also cross-react with cardiac MLC-1 (CMLC-1). The assay provides an excellent tool for assessment of skeletal muscle injury in the absence of cardiac damage.

PRINCIPLE

The **K-ASSAY®** Rat & Mouse Skeletal Muscle Myosin Light Chain-1 ELISA uses two different MLC-1 monoclonal antibodies. One is used for solid phase immobilization (on the microtiter wells). The second is conjugated to horse radish peroxidase (HRP) and is used for detection. The sample is diluted with diluent as necessary and 100 µL aliquots of samples and calibrators are incubated in the microtiter wells for one hour. The wells are then washed and anti-MLC-1 HRP conjugate is added and incubated in the wells for one hour. MLC-1 molecules are thereby sandwiched between the solid phase and HRP-conjugated antibodies. After washing to remove unbound HRP conjugate a solution of tetramethylbenzidine (TMB), an HRP substrate, is then added to the wells and incubated for 20 minutes, resulting in the development of a blue color. Color development is stopped by the addition of 1N HCl changing the color to yellow. The concentration of MLC-1 is proportional to the absorbance at 450 nm and is derived from a calibration curve.

COMPONENTS

- Anti-MLC-1-coated microtiter wells, 96 wells (12 x 8-well strips)
- MLC-1 Calibrator (lyophilized)
- Diluent, 25 mL
- MLC-1 HRP Conjugate, 11 mL
- Wash Solution (20X), 50 mL
- TMB Reagent, 11 mL
- Stop Solution, 11 mL

MATERIALS REQUIRED BUT NOT PROVIDED

- Pipettes: P-10, P-200 & P-1000 or equivalent
- Disposable pipette tips
- Distilled or de-ionized water
- Vortex mixer
- Absorbent paper
- Graph paper or appropriate PC graphing software
- Polypropylene microcentrifuge tubes (1.5 mL)
- Microtiter plate reader capable of reading OD at 450 nm.

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. Equilibrate kit components to room temperature before use.
2. Reconstitute the lyophilized MLC-1 stock as directed on the vial label. Mix gently several times over a period of 5-10 minutes.
3. Label 6 polypropylene tubes as 50, 25, 12.5, 6.25, 3.125 and 1.56 ng/mL.
4. Into the tube labeled 50 ng/mL, pipette the volume of diluent detailed on the MLC-1 stock vial label. Then add the indicated volume of MLC-1 stock (shown on the vial label) and mix gently. This provides the 50 ng/mL calibrator.
5. Pipette 0.25 mL of calibrator diluent into the tubes labeled 25, 12.5, 6.25, 3.125 and 1.56 ng/mL.
6. Prepare a 25 ng/mL calibrator by diluting and mixing 0.25 mL of the 50 ng/mL calibrator with 0.25 mL of diluent in the tube labeled 25 ng/mL. Similarly prepare the 12.5, 6.25, 3.125 and 1.56 ng/mL calibrators by serial dilution.

NOTE: The reconstituted MLC-1 stock should be frozen immediately after use. It remains stable in frozen form for at least 6 months at -70°C. Discard the working 50 – 1.56 ng/mL calibrators after use.

SAMPLE COLLECTION AND 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 prior to use. **We recommend that samples be assayed in duplicate. Optimum sample dilution should be determined by the end user. Samples should only be diluted with the diluent supplied with the kit.**

PROCEDURAL NOTES

1. Calibrators and diluted plasma samples should be prepared immediately prior to use and should be used within 30 minutes.
2. Pipetting of conjugate, calibrators and samples into the microtiter plate should be completed within 5 minutes.
3. It is recommended that the wells be read within 5 minutes following addition of Stop Solution.

ASSAY PROCEDURE

1. Secure the desired number of coated wells in the holder.
2. Dispense 100 µL of calibrators and diluted samples into the appropriate wells.
3. Thoroughly mix and incubate on an orbital micro-plate shaker at 150 rpm at room temperature (18-25°C) for one hour.
4. Remove the incubation mixture using either a plate washer or by flicking plate contents into an appropriate Bio-waste container.
5. Wash and empty the microtiter wells 5 times with 1x wash solution preferably using a plate washer (400 µL/well). Alternatively, a squirt bottle may be used. The entire wash procedure should be performed as quickly as possible.
6. Strike the wells sharply onto absorbent paper or paper towels to remove all residual droplets.
7. Add 100 µL of enzyme conjugate reagent into each well.
8. Incubate on an orbital micro-plate shaker at 150 rpm at room temperature (18-25°C) for one hour.
9. Wash as detailed in 4 to 6 above.
10. Dispense 100 µL of TMB Reagent into each well.
11. Gently mix on an orbital micro-plate shaker at ~150 rpm at room temperature (18-25°C) for 20 minutes.
12. 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 the optical density at 450 nm with a microtiter plate reader *within 5 minutes*. Due to plate reader differences, the high calibrator absorbance values may occasionally be out of range. If this occurs, absorbance values may be determined at 405 nm instead.
15. If absorbance values of samples exceed those of the highest calibrator, samples should be further diluted with diluent and re-tested. For practical purposes, samples with absorbance values below that of the lowest calibrator should be assigned a zero value.

CALCULATION OF RESULTS

1. Calculate the mean absorbance value (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.

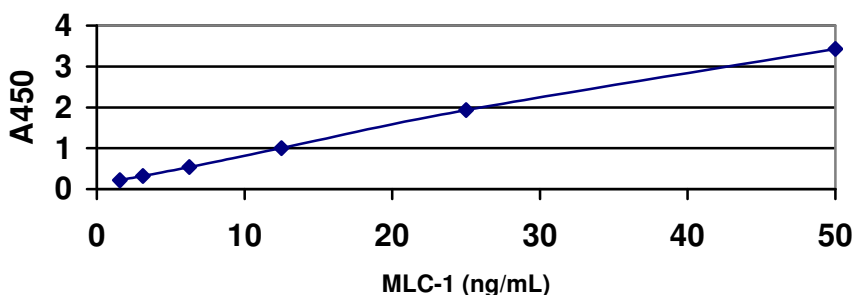
- Using the A_{450} values for each sample, determine the corresponding concentration of MLC-1 (ng/mL) from the calibration curve. If using graphing software, we suggest using a point-to-point, or two site binding (hyperbola) fit of the data. The end user should choose the best data fit for the calibration curve.
- Multiply the derived MLC-1 concentrations by the dilution factor to obtain the actual MLC-1 concentration.

TYPICAL CALIBRATION CURVE

Results of a typical calibration run with optical density reading at 450 nm shown on the Y axis against MLC-1 concentration shown on the X axis are illustrated below. This calibration curve is for the illustration purpose only and should not be used to calculate unknowns. A calibration curve should be run for each assay.

MLC-1 (ng/mL)	Absorbance (450 nm)
50	3.433
25	1.937
12.5	1.000
6.25	0.538
3.13	0.317
1.56	0.211

Typical MLC-1 Calibration Curve



STORAGE

Store the kit at 4 °C. Keep the microtiter plate in a sealed bag with desiccant to minimize exposure to damp air. The expiration date of the kit is indicated on the box label.

WARNINGS AND PRECAUTIONS

- 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.
- Do not use reagents after expiration date and do not mix or use components from kits with different lot numbers.
- Replace caps on reagents immediately. Do not switch caps.
- Do not pipette reagents by mouth.

LIMITATIONS OF THE PROCEDURE

- Reliable and reproducible results will be obtained when the assay procedure is carried out with a complete understanding of the package insert 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.

FOR RESEARCH USE ONLY

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