RAT & MOUSE SKELETAL MUSCLE MYOSIN LIGHT CHAIN-1 ELISA
Life Diagnostics, Inc., Catalog Number: SMLC-2

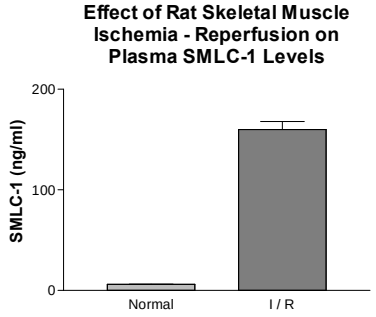
Rat and Mouse Skeletal Muscle Myosin Light Chain-1 (SMLC-1) ELISA

STORAGE CONDITIONS

Store the kit at 2-8°C. Keep the microtiter plate in a sealed bag with desiccant to minimize exposure to damp air.

BACKGROUND

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). However, as indicated in the figure below, the assay provides an excellent tool for assessment of skeletal muscle injury in the absence of cardiac damage.[1]



PRINCIPLE OF THE ASSAY

The SMLC-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 horseradish peroxidase (HRP) and is used for detection. The samples are diluted with diluent as necessary and 100 □l of samples and standards are incubated in the microtiter wells for one hour. The wells are then washed, and anti-SMLC-1 HRP conjugate is added and incubated in the wells for one hour. SMLC-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. The color development is stopped by addition of 1N HCl, changing the color to yellow. The concentration of SMLC-1 is proportional to the absorbance at 450 nm and is derived from a standard curve.

MATERIALS AND COMPONENTS

Reagents and materials provided

- Anti MLC-1-Coated Wells (1 plate, 96 wells, 12 x 8-well strips)
- SMLC-1 Stock: Lyophilized rat SMLC-1
- Diluent (25 ml)
- 20x Wash Solution (50 ml)
- SMLC-1 HRP Conjugate (11 ml)
- TMB Reagent (11 ml)
- Stop Solution (11 ml)

Materials required but not provided

- Distilled or deionized water
- Pipettes: P-10, P-200 & P-1000 or equivalent
- Disposable pipette tips
- Microtiter well reader capable of reading OD at 450 nm
- Vortex mixer
- Absorbent paper
- Graph paper or appropriate PC graphing software
- Polypropylene microcentrifuge tubes (1.5 ml)
- Plate shaker with mixing speed of ~150 rpm

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 deionized water.

STANDARD PREPARATION

1. Equilibrate kit components to room temperature before use.
2. Reconstitute the lyophilized SMLC-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 SMLC-1 stock vial label. Then add the indicated volume of SMLC-1 stock (shown on the vial label) and mix gently. This provides the 50 ng/ml standard.
5. Pipette 0.25 ml of standard diluent into the tubes labeled 25, 12.5, 6.25, 3.125 and 1.56 ng/ml.
6. Prepare a 25 ng/ml standard by diluting and mixing 0.25 ml of the 50 ng/ml standard 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 standards by serial dilution.

NOTE: The reconstituted SMLC-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 standards 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 ï 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 diluent supplied with the kit.

ASSAY PROCEDURE

1. Secure the desired number of coated wells in the holder.
2. Dispense 100 µl of standards and diluted samples into appropriate wells.
3. Thoroughly mix and incubate on an orbital shaker (150 rpm) at room temperature (18-25°C) for one hour.

[1] Cardiac injury may be assessed using rat or mouse cardiac troponin-I ELISA kits available from Life Diagnostics, Inc.

4. Remove the incubation mixture using a plate washer or by flicking the plate contents into a waste container.
5. Wash and empty the microtiter wells 5 times with 1x wash solution. This should preferably be performed with 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 wash buffer.
7. Dispense 100 µl of SMLC-1 HRP conjugate into each well.
8. Incubate on an orbital shaker (150 rpm) at room temperature (18-25°C) for one hour.
9. Wash the microtiter wells as directed in steps 4 -6 above.
10. Dispense 100 µl of TMB Reagent into each well.
11. Incubate at room temperature for 20 minutes on an orbital shaker at ~150 rpm.
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 absorbance at 450 nm with a microtiter well reader within 5 minutes. *Please Note:* ***Due to plate reader differences, the high standard absorbance values may occasionally be out of range. If this occurs, absorbance values may be determined at 405 nm instead.***
15. If the absorbance values of the samples exceed those of the highest standard, the samples should be further diluted with diluent and re-tested. For practical purposes, samples with absorbance values below that of the lowest standard should be assigned a zero value.

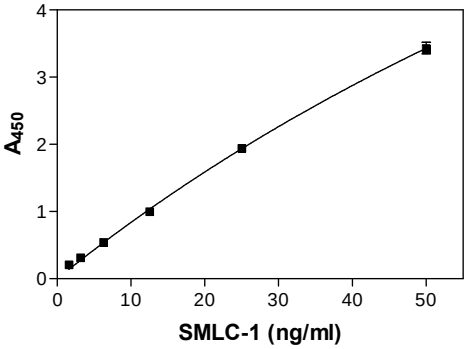
CALCULATION OF RESULTS

1. Calculate the mean absorbance value (A_{450}) for the standards and samples.
2. Construct a standard curve by plotting the A_{450} values obtained for each reference standard against its concentration in ng/ml on graph paper, with absorbance on the vertical (y) axis and concentration on the horizontal (x) axis.
3. Using the A_{450} values for each sample, determine the corresponding concentration of SMLC-1 (ng/ml) from the standard curve. If using graphing software, we suggest a point-to-point or two site binding (hyperbola) fit of the data. The end user should choose the best data fit for the standard curve.
5. Multiply the derived SMLC-1 concentrations by the dilution factor to obtain the actual SMLC-1 concentration.

TYPICAL STANDARD CURVE

Results of a typical standard curve with A_{450} plotted on the Y axis against SMLC-1 concentrations on the X axis are shown below. **NOTE:** This standard curve is for the purpose of illustration only.

SMLC-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



PROCEDURAL NOTES

1. Standards should be prepared immediately prior to use and should be used within 30 minutes of preparation.
2. Pipetting of conjugate, standards and samples into the microtiter plate should be completed within 10 minutes.
3. We recommend that standards and samples be run in duplicate.
4. It is recommended that the wells be read within 5 minutes following addition of Stop Solution.
5. Reliable and reproducible results will be obtained when the assay procedure is carried out with a complete understanding of the package insert instructions.
6. The wash procedure is critical. Insufficient washing will result in poor precision and falsely elevated absorbance readings

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For technical assistance please email us at
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