RAT MYOGLOBIN ELISA
Catalog Number: MYO-2

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INTRODUCTION

Myoglobin is a 17 kDa heme protein expressed in cardiac and skeletal muscle. After cardiac injury, it is one of the first biomarkers to rise above normal levels. In humans, it increases above baseline within 2-4 hours, peaking at 9-12 hours, and returning to baseline within 24-36 hours. In the absence of skeletal muscle injury, it can used as a biomarker for cardiac muscle injury. Similarly, in the absence of cardiac damage, myoglobin can be used as a biomarker of skeletal muscle injury.

PRINCIPLE OF THE ASSAY

The assay uses a monoclonal myoglobin antibody for solid phase immobilization (microtiter wells) and horseradish peroxidase (HRP) conjugated polyclonal myoglobin antibody for detection. Standards and diluted samples are co-incubated with HRP conjugate in the microtiter wells for one hour. The wells are then washed to remove unbound HRP-conjugate. TMB is added and incubated for 20 minutes. If myoglobin 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 myoglobin is proportional to absorbance and is derived from a standard curve.

MATERIALS AND COMPONENTS

Materials provided with the kit:

- Antibody coated 96-well plate (12 x 8-well strips)
- HRP Conjugate, 11 ml
- Myoglobin stock, 50 µg/ml
- 20x Wash Solution: TBS50-20, 50 ml
- Diluent: YD12-1, 12 ml
- TMB: TMB11-1, 11 ml
- Stop Solution: SS11-1, 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 myoglobin stock in a -20°C freezer. The remainder of the kit should be stored at 4°C. The microtiter plate should be kept in a sealed bag with desiccant. Kits will remain stable for six months from the date of purchase.

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. The ELISA kit was calibrated using a shaking incubator 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 deionized water.

STANDARD PREPARATION

1. Label 8 polypropylene or glass tubes as 100, 50, 25, 12.5, 6.25, 3.125, 1.56 and 0 ng/ml.
2. Pipette 998 µl of diluent into the tube labelled 100 ng/ml and 100 µl of diluent into the remaining tubes.
3. Briefly centrifuge or flick the myoglobin stock to ensure that the liquid contents are at the bottom of the tube.
4. Mix 2.0 µl of 50 µg/ml myoglobin stock with 998 µl of diluent to give the 100 ng/ml standard.
5. Prepare the 50 ng/ml standard by mixing 100 µl of the 100 ng/ml standard with 100 µl of diluent in the tube labeled 50 ng/ml.
6. Similarly prepare the 25, 12.5, 6.25, 3.125 and 1.56 ng/ml standards by two-fold serial dilution.

SAMPLE COLLECTION

Serum, plasma and urine should be collected using standard techniques. Plasma samples should be collected into tubes containing EDTA. Samples that cannot be assayed within 3 hours of collection should be frozen at -20°C or lower. Samples should not be repeatedly frozen and thawed.

SAMPLE PREPARATION

Serum samples can be tested undiluted or after dilution with diluent YD12-1. The dilution factor should be determined empirically. A matrix effect may occur with urine samples at low dilutions. We recommend that all urine samples in a study be similarly diluted.

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. Pipette 100 µl of HRP conjugate into the wells.
3. Add 20 µl of samples and standards into the appropriate wells. We recommend that standards and samples be tested in duplicate.
4. Incubate on a plate shaker at 150 rpm and 25°C 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 absorbent 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 at 25°C 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.

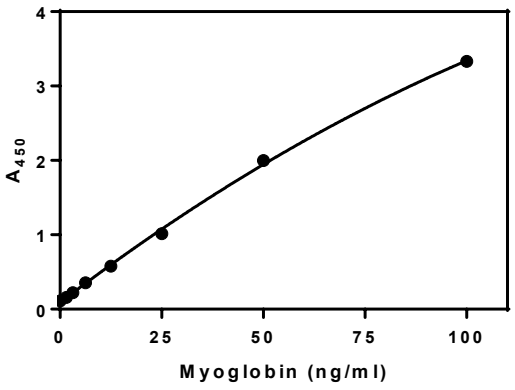
CALCULATION OF RESULTS

1. Using curve fitting software, construct a standard curve by plotting absorbance values of the standards versus concentration.
2. Fit the standard 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, if applicable, to determine the actual concentration in the sample.
4. If the A_{450} values of samples fall outside the standard curve, samples should be diluted appropriately and re-tested.

TYPICAL STANDARD CURVE

A typical standard curve is shown below. This curve is for illustration only and should not be used to calculate unknowns. A standard curve should be generated for each experiment.

Myoglobin (ng/ml)	A_{450}
100	3.333
50	1.997
25	1.015
12.5	0.577
6.25	0.356
3.125	0.223
1.563	0.158
0	0.110



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