RAT MYOGLOBIN ELISA
Life Diagnostics, Inc., Catalog Number: 2110-2-N

ELISA for the Determination of Myoglobin in Rat Serum, Plasma & Urine[1]

STORAGE

Store standard at -20°C
STORE REMAINDER OF KIT AT 2 - 8°C

INTRODUCTION

Myoglobin is a heme protein found in both cardiac and skeletal muscle. Following myocardial necrosis associated with myocardial infarction (MI), myoglobin is one of the first markers to rise above normal levels. Studies with human subjects have shown that myoglobin increases measurably above baseline within 2-4 hours post-infarct, peaking at 9-12 hours, and returning to baseline within 24-36 hours. In the absence of skeletal muscle trauma or other factors associated with a noncardiac related increase in circulating myoglobin, myoglobin may used as a marker for MI. Similarly, in the absence of cardiac damage, myoglobin may be used as a marker of skeletal muscle injury.

PRINCIPLE OF THE TEST

The Myoglobin ELISA is based on the principle of a solid phase enzyme-linked immunosorbent assay. A monoclonal anti-myoglobin antibody is used for solid phase immobilization (on the microtiter wells) and a polyclonal anti-myoglobin antibody conjugated to horseradish peroxidase is in the antibody-enzyme conjugate solution. The test sample is allowed to react simultaneously with the two antibodies, resulting in the myoglobin molecules being sandwiched between the solid phase and enzyme-linked antibodies. After one hour incubation at room temperature, the wells are washed to remove unbound labeled antibodies. A TMB (Tetramethyl-benzidine) Reagent is added and incubated for 20 minutes, resulting in the development of a blue color. The color development is stopped with the addition of Stop Solution changing the color to yellow. The concentration of myoglobin is proportional to the color intensity of the test sample. Absorbance is measured spectrophotometrically at 450 nm.

REAGENTS

Materials provided with the kit:

- Anti-Myoglobin-coated microtiter wells, 96 wells
- Rat Myoglobin Stock (50 µl of 50 µg/ml) **Store at -20°C**
- Diluent, 12 ml
- Enzyme Conjugate Reagent, 11 ml
- 20x Wash Solution, 50 ml
- TMB Reagent (One-Step), 11 ml
- Stop Solution (1N HCl), 11 ml

[1] A matrix effect may be observed with urine samples that results in slight differences in absorbance values relative to myoglobin diluted in the ELISA diluent. It is therefore recommended that wherever possible all urine samples within a particular study be similarly diluted prior to testing, thereby ensuring an accurate determination of relative myoglobin levels within the study.

Materials required but not provided:

- Precision pipettes
- Disposable pipette tips
- Distilled or de-ionized water
- Vortex mixer
- Absorbent paper or paper towels
- Graph paper (PC graphing software is optional but recommended)
- Plate shaker
- Microtiter plate reader

INSTRUMENTATION

A microtiter plate reader with an optical density range of 0-4 OD at 450 nm wavelength is required.

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 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 labeled 100 ng/ml
3. Pipette 100 µl of diluent into the tubes labeled 50, 25, 12.5, 6.25, 3.125, 1.56 and 0 ng/ml.
4. Briefly centrifuge or flick the myoglobin stock tube to ensure that the liquid contents are at the bottom of the tube.
5. Dilute 2 µl of the 50 µg/ml myoglobin stock into the 998 µl of diluent in the tube labeled 100 ng/ml. This provides a 100 ng/ml solution of myoglobin.
6. Prepare a 50 ng/ml stock by diluting and mixing 100 µl of the 100 ng/ml stock with 100 µl of diluent in the tube labeled 50 ng/ml. Similarly prepare the 25, 12.5, 6.25, 3.125, 1.56 ng/ml stocks by serial dilution.

SAMPLE COLLECTION

Serum, plasma and urine should be collected using standard techniques. Remove serum or plasma from the coagulated or packed cells within 60 minutes after collection. Plasma samples may 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 prior to testing.

SAMPLE PREPARATION

Samples may be tested undiluted or after dilution with diluent. The dilution factor should be determined empirically. On occasion a matrix effect may be observed with urine samples that may slightly increase or decrease absorbance values and we therefore strongly recommend that all urine samples within a particular study be similarly diluted. *Only 20 µl of*

sample is required per assay (2 x 20 µl, if samples are to be tested in duplicate).

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.

ASSAY PROCEDURE

1. Ensure that all reagents are at room temperature.
2. Secure the desired number of coated wells in the holder.
3. Dispense 100 µl of Enzyme Conjugate Reagent into each well.
4. Dispense 20 µl of myoglobin standards and samples (in duplicate) into the appropriate wells.
5. Incubate at room temperature (18-25°C) on a plate shaker for one hour. Mix Gently (~100-150 rpm)
6. Remove the incubation mixture either with a plate washer or by flicking plate contents into a waste container.
7. Wash and empty the microtiter wells 5 times with 1x wash solution. This may performed using either a plate washer (400 µl/well) or with a squirt bottle. The entire wash procedure should be performed as quickly as possible.
8. Strike the wells sharply onto absorbent paper or paper towels to remove all residual wash buffer.
9. Dispense 100 µl of TMB Reagent solution into each well. Gently mix for 5 seconds.
10. Incubate on a plate shaker at room temperature for 20 minutes. Mix Gently
11. Stop the reaction by adding 100 µl of Stop Solution to each well.
12. Gently mix 30 seconds. It is important to make sure that all the blue color changes to yellow color completely.
13. Read absorbance at 450 nm with a microtiter well reader within 5 minutes.

CALCULATION OF RESULTS

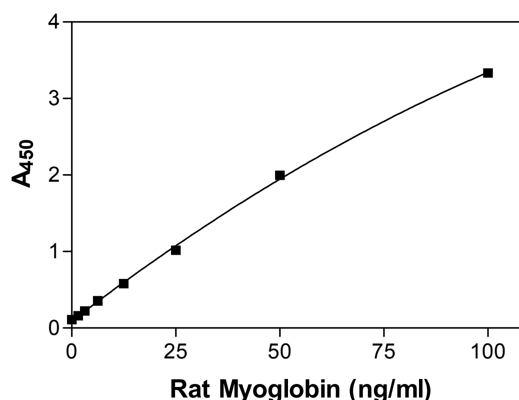
1. Calculate the mean absorbance value for each set of reference standards and samples.
2. Construct a standard curve by plotting the mean absorbance obtained for each reference standard against its concentration in ng/ml on graph paper, with absorbance values on the vertical or Y axis, and concentrations on the horizontal or X axis.
3. Use the mean absorbance values for each sample to determine the corresponding concentration of myoglobin in ng/ml from the standard curve.
4. Multiply the derived value by the appropriate dilution factor if the test samples were diluted.
5. Graphing software, if available, should be used.

TYPICAL STANDARD CURVE

Results of a typical standard run with optical density readings at 450 nm shown in the Y axis against myoglobin concentrations shown in the X axis are illustrated below. This standard curve is for illustrative purposes only, and should not be used to calculate unknowns. A standard curve should be run for each assay.

Myoglobin (ng/ml)	Absorbance (450 nm)
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

Typical Rat Myoglobin Standard Curve



LIMITATIONS OF THE PROCEDURE

1. Reliable and reproducible results will be obtained when the assay procedure is carried out with a complete understanding of the package insert instructions.
2. The wash procedure is critical. Insufficient washing will result in poor precision and falsely elevated absorbance readings.