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# MYOGLOBIN ENZYME IMMUNOASSAY TEST KIT

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## Enzyme Immunoassay for the Quantitative Determination of Myoglobin Concentration in Human Serum or Plasma

### FOR RESEARCH USE ONLY

Store at 2 to 8°C.

### PROPRIETARY AND COMMON NAMES

Human Myoglobin Enzyme Immunoassay

### 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, increasing measurably above baseline within 2-4 hours post-infarct, peaking at 9-12 hours, and returning to baseline within 24-36 hours.<sup>1,2</sup> In the absence of skeletal muscle trauma or other factors associated with a noncardiac related increase in circulating myoglobin, myoglobin levels have been used as an early marker for MI.<sup>3,4,5</sup> A number of reports suggest the measurement of myoglobin as a diagnostic aid in "ruling-out" myocardial infarction<sup>6,7</sup> with negative predictive values of up to 100% reported at certain time periods after onset of symptoms.<sup>8</sup>

### PRINCIPLE OF THE TEST

The Myoglobin ELISA test is based on the principle of a solid phase enzyme-linked immunosorbent assay.<sup>9,10</sup> The assay system utilizes a unique monoclonal antibody directed against a distinct antigenic determinant on the myoglobin molecule. Mouse monoclonal anti-myoglobin antibody is used for solid phase immobilization (on the microtiter wells). A goat anti-myoglobin antibody is in the antibody-enzyme (horseradish peroxidase) 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 a 45 minute incubation at room temperature, the wells are washed with water to remove unbound labeled antibodies. A TMB (Tetramethylbenzidine) 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 directly proportional to the color intensity of the test sample. Absorbance is measured spectrophotometrically at 450 nm.

### REAGENTS

#### *Materials provided with the kit:*

- Murine Monoclonal Anti-Myoglobin-coated microtiter wells, 96 wells
- Myoglobin Reference Standards: 0, 25, 100, 250, 500 and 1000 ng/ml. Liquid, 1 ml each. 1 set. ***These standards have been pre-diluted 10-fold. Please do not dilute them again.***
- Sample Diluent, 25 ml
- Enzyme Conjugate Reagent, 22 ml
- TMB Reagent (One-Step), 11 ml
- Stop Solution (1N HCl), 11 ml

#### *Materials required but not provided:*

- Precision pipettes: 20 µl, 50 µl, 200 µl, and 1.0 ml
- Disposable pipette tips
- Distilled water
- Vortex mixer or equivalent.
- Absorbent paper or paper towels
- Graph paper
- Microtiter plate reader

### SPECIMEN COLLECTION AND PREPARATION

Specimens may be collected using standard venipuncture 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. The use of freshly collected (within 24 hours) samples is recommended. Specimens which cannot be assayed within 24 hours of collection should be frozen at -20°C or lower for up to six months.

Specimens should not be repeatedly frozen and thawed prior to testing. DO NOT store in "frost free" freezers, which may cause occasional thawing. Specimens which have been frozen, and those which are turbid and/or contain particulate matter, must be centrifuged prior to use.

### WARNINGS AND PRECAUTIONS FOR USERS

Test methods are not available which can offer complete assurance that Hepatitis B virus, Human Immunodeficiency Virus (HIV/HTLV-III/LAV), or other infectious agents are absent from the reagents in this kit. Therefore, all human products, including patient samples, should be considered potentially infectious. Handling and disposal should be in accordance with the procedures defined by an appropriate national biohazard safety guideline or regulation, where it exists (e.g., USA Center for Disease Control/National Institute of Health Manual, "Biosafety in Microbiological and Biomedical Laboratories," 1984).<sup>11</sup>

## STORAGE OF TEST KIT AND INSTRUMENTATION

Unopened test kits should be stored at 2-8°C upon receipt and the microtiter plates should be kept in a sealed bag with desiccants to minimize exposure to damp air. Opened test kits will remain stable until the expiration date shown, provided they are stored as described above. A microtiter plate reader with a bandwidth of 10 nm or less and an optical density range of 0-2 OD or greater at 450 nm wavelength is acceptable for use in absorbance measurement.

## REAGENT PREPARATION

1. All reagents should be brought to room temperature (18-25°C) before use.
2. ***Patient serum and control serum should be diluted 10 fold before use. Prepare a series of small tubes (such as 1.5 ml microcentrifuge tubes) and mix 20 µl serum with 180 µl (0.18 ml) Sample Diluent. PLEASE DO NOT DILUTE THE STANDARDS – THEY HAVE ALREADY BEEN PRE-DILUTED 10-FOLD.***
3. Samples with expected myoglobin concentrations over 1000 ng/ml may be quantitated by further dilution 10-fold with sample diluent.

## ASSAY PROCEDURE

1. ***Serum should be diluted 10 fold before use. Prepare a series of small tubes (such as 1.5 ml microcentrifuge tubes) and mix 20 µl serum or plasma with 180 µl (0.18 ml) Sample Diluent. PLEASE DO NOT DILUTE THE STANDARDS – THEY HAVE ALREADY BEEN PRE-DILUTED 10-FOLD.***
2. Secure the desired number of coated wells in the holder.
3. Dispense 20 µl of myoglobin standards, diluted specimens and diluted controls into the appropriate wells.
4. Dispense 200 µl of Enzyme Conjugate Reagent into each well.
5. Thoroughly mix for 30 seconds. It is very important to mix completely.
6. Incubate at room temperature (18-25°C) for 45 minutes.
7. Remove the incubation mixture by flicking plate contents into a waste container.
8. Rinse and flick the microwells 5 times with distilled or deionized water. (Please do not use tap water.)
9. Strike the wells sharply onto absorbent paper or paper towels to remove all residual water drops.
10. Dispense 100 µl of TMB Reagent solution into each well. Gently mix for 5 seconds.
11. Incubate at room temperature for 20 minutes.
12. Stop the reaction by adding 100 µl of Stop Solution to each well.
13. Gently mix 30 seconds. ***It is important to make sure that all the blue color changes to yellow color completely.***
14. Read absorbance at 450 nm with a microtiter well reader **within 15 minutes.**

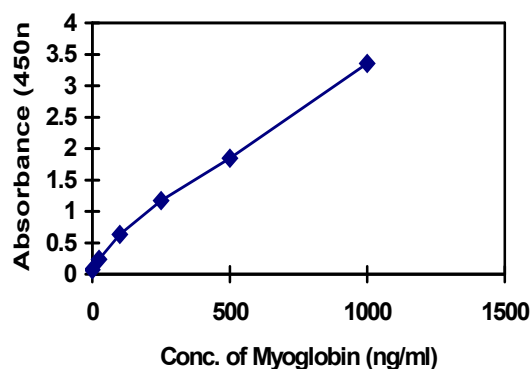
## CALCULATION OF RESULTS

1. Calculate the mean absorbance value ( $A_{450}$ ) for each set of reference standards, controls 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 specimen to determine the corresponding concentration of myoglobin in ng/ml from the standard curve.
4. Since the reference standards have already been pre-diluted 10-fold, there is no need for the patient samples or control sera observed values to be multiplied by the dilution factor of 10. However, if the patient samples are diluted to 100-fold, the observed values should be multiplied by 10.

## EXAMPLE OF 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. This standard curve is for illustrative purposes only, and should not be used to calculate unknowns. Each laboratory should obtain its own data and standard curve.

Myoglobin (ng/ml)	Absorbance (450 nm)
0	0.071
25	0.235
100	0.632
250	1.169
500	1.845
1000	3.357



## EXPECTED NORMAL VALUES

Normal serum myoglobin levels range from 12 to 90 ng/ml. Values increase slightly with age.

Myoglobin can be used as a diagnostic aid in "ruling-out" myocardial infarction<sup>6,7</sup> with negative predictive values of up to 100% reported at certain time periods after onset of symptoms.<sup>8</sup>

Each facility should establish its own reference intervals for myoglobin as performed on the ELISA test. Other factors should also be considered in the diagnosis of myocardial infarction.

## SENSITIVITY

The lowest detectable level of myoglobin by this assay is estimated to be 5 ng/ml.

## HOOK EFFECT

No high-dose hook effect is observed in this test with patient sample concentrations up to 100,000 ng/ml.

## 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 and with adherence to good laboratory practice.
2. The wash procedure is critical. Insufficient washing will result in poor precision and falsely elevated absorbance readings.
3. The results obtained from the use of this kit should be used only as an adjunct to other diagnostic procedures and information available to the physician.
4. Samples may contain human anti-mouse antibodies (HAMA) which are capable of giving falsely elevated or depressed results with assays that utilize mouse monoclonal antibodies. This assay has been designed to minimize interference from HAMA-containing specimens. Nevertheless, complete elimination of this interference from all patient specimens cannot be guaranteed. A test result that is inconsistent with the clinical picture and patient history should be interpreted with caution.

## REFERENCES

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