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## Myoglobin ELISA

Catalog No.: BQ017C (96 tests)

**INTENDED USE**

The Myoglobin ELISA is intended for the quantitative determination of myoglobin in human serum.

**SUMMARY AND EXPLANATION**

Myoglobin, a heme protein with a molecular weight of approximately 17,500 Daltons is found in both cardiac and skeletal muscle. Damage to either type of muscle following conditions such as trauma, ischemia, and diseases that cause myopathy, is associated with the release of myoglobin into serum. Specifically, following cardiac necrosis associated with myocardial infarction (MI), myoglobin is one of the first markers to rise above normal levels. Myoglobin levels increase 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 non-cardiac related increase in circulating myoglobin, its levels have been used as an early marker for myocardial infarct. A number of reports suggest using the measurement of myoglobin as a diagnostic aid in ruling out myocardial infarction with negative predictive values of up to 100% reported at certain time periods after the onset of symptoms. 9-15 Unlike the other cardiac enzymes such as creatine kinase and the MB isoform (i.e., CK and CK/MB) which do not reach serum levels until several hours post-infarction (approx. 19 hours), myoglobin levels can be expected to peak within 6 to 9 hours. The Myoglobin Enzyme Immunoassay provides a rapid, sensitive, and reliable assay for the quantitative measurement of myoglobin in serum. The antibodies developed for the test will determine a minimal concentration of 5.0 ng/ml, and there is no cross-reactivity with related cardiac or skeletal enzymes.

**PRINCIPLE OF THE TEST**

The Myoglobin ELISA test is based on the principle of a solid phase enzyme-linked immunosorbent assay. 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 (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 directly proportional to the color intensity of the test sample. Absorbance is measured spectrophotometrically at 450 nm.

MATERIALS PROVIDED	96 tests
Microwell coated with murine monoclonal anti-myoglobin.	12x8x1
Reference Standard Set	1 ml
Sample Diluent	25 ml
Enzyme Conjugate Reagent	22 ml
TMB Reagent	11 ml
Stop Solution	11 ml

**MATERIALS NOT PROVIDED**

- Distilled or deionized water
- Precision pipettes
- Disposable pipette tips
- ELISA reader capable of reading absorbance at 450 nm
- Absorbance paper or paper towel
- Graph paper

**STORAGE AND STABILITY**

1. Store the kit at 2 – 8° C.
2. Keep microwells sealed in a dry bag with desiccants.
3. The reagents are stable until expiration of the kit.
4. Do not expose test reagents to heat, sun or strong light

**WARNINGS AND PRECAUTIONS**

1. Potential biohazardous materials: The calibrator and controls contain human source components, which have been tested and found non-reactive for hepatitis B surface antigen as well as HIV antibody with FDA licensed reagents. However, as there is no test method that can offer complete assurance that HIV, Hepatitis B virus or other infectious agents are absent, these reagents should be handled at the Biosafety Level 2, as recommended in the Centers for Disease Control/National Institutes of Health manual, "Biosafety in Microbiological and Biomedical Laboratories." 1984
2. This kit is designed for Research Use Only.
3. Optimal results will be obtained by strict adherence to the test protocol. Precise pipetting as well as following the exact time and temperature requirements is essential.
4. Do not pipette by mouth. Do not smoke, eat, or drink in the areas in which specimens or kit reagents are handled.
5. The components in this kit are intended for use as an integral unit. The components of different lots should not be mixed.
6. This product contains components preserved with sodium azide. Sodium azide may react with lead and copper plumbing to form explosive metal azide. On disposal, flush with a large volume of water.

**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. 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 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.

**PERFORMANCE CHARACTERISTICS**

Correlation coefficient = 0.9392, Slope = 0.9948, Intercept = 55.051, The Mean = 287.9 ng/ml, Abbott Myoglobin Mean = 262.5 ng/ml

**1. SENSITIVITY**

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

**2. PRECISION****Intra-Assay Precision**

Within-run precision was determined by replicate determinations of five different serum samples in one assay. Within-assay variability as shown:

Serum Sample	1	2	3	4	5
# Reps.	2 0	2 0	2 0	2 0	2 0
Mean Myo. (ng/ml)	5 5. 6	2 1 4.	2 9 9	5 0 5. 9	1, 4 3 7
S.D.	2. 2	1 2. 9	1 6. 2	2 6. 3	9 4. 0
C.V. (%)	3. 9 %	6. 0 %	5. 5 %	5. 2 %	6. 6 %

**3. Inter-Assay Precision**

Between-run precision was determined by replicate measurements of five different serum samples over a series of individually calibrated assays. Between-assay variability is shown below:

Serum Sample	1	2	3	4	5
# Replicates	3 5	35	35	35	35
Mean Myo (ng/ml)	5 9 .2	24 4.4	33 0.5	56 8.3	1451 .7
S.D.	4 .6	12. 8	38. 9	52. 7	104. 7
C.V. (%)	7 .8 %	5.2 %	11. 8%	9.3 %	7.2%

**4. RECOVERY**

Various patient serum samples of known myoglobin levels were combined and assayed in duplicate. The mean recovery was 102.8%.

PAI R NO.	EXPECTED [Myoglobin] (ng/ml)	OBSERVED [Myoglobin] (ng/ml)	% RECOVER Y
1	280	250	89.3%
2	451	495	109.8%
3	255	241	94.5%
4	269	300	111.5%
5	39	41	105.1%
6	240	231	96.0%
7	92	88	95.9%
8	209	214	102.0%
9	340	328	96.0%
10	214	213	100.0%
11	551	655	118.8%
12	431	436	101.2%
13	757	824	108.8%
14	747	768	102.8%
15	780	894	114.6%
16	575	569	98.9%