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Product Information

Myoglobin ELISA kit

Catalog Number: EA100895
Storage Temperature: 2 – 8°C

Instruction for Use

Intended Use

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

Background

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 creeatine 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.

Components

MATERIALS PROVIDED	96 Tests
1. Microwell coated with murine monoclonal anti-myoglobin.	12x8x1
2. Reference Standard Set	0.5 ml
3. Sample Diluent	25 ml
4. Enzyme Conjugate Reagent	22 ml
5. TMB Reagent	11 ml
6. Stop Solution	11 ml
7. Wash Concentrate 20x: 1 Bottle	25 ml

Materials and Equipment Required but Not Provided

1. Distilled or deionized water
2. Precision pipettes
3. Disposable pipette tips
4. ELISA reader capable of reading absorbance at 450nm
5. Absorbance paper or paper towel
6. Graph paper

Disclaimer

This product is for research use only and not intended for diagnostic procedures.

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 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.
4. Prepare 1X Wash buffer by adding the contents of the bottle (25 ml, 20X) to 475 ml of distilled or deionized water. Store at room temperature (18-26°C).

Assay Procedure

- Before proceeding with the assay, bring all reagents, serum references and controls to room temperature (18-26°C). Gently mix all reagents before use
- The components in this kit are intended for use as an integral unit. The components of different lots should not be mixed
- It is recommended that standards, control and serum samples be run in duplicate
- Do not use sodium azide as preservative. Sodium azide inhibits HRP enzyme activities

1. Secure the desired number of coated wells in the holder.
2. Dispense 20 µl of myoglobin standards, diluted specimens and diluted controls into the appropriate wells.
3. Dispense 200 µl of Enzyme Conjugate Reagent into each well.
4. Thoroughly mix for 30 seconds. It is very important to mix completely.
5. Incubate at room temperature (18-25°C) for 45 minutes.
6. Remove the incubation mixture by flicking plate contents into a waste container.

7. Remove liquid from all wells. Wash wells three times with 300 µl of 1X wash buffer. Blot on absorbance paper or paper towel.
8. Strike the wells sharply onto absorbent paper or paper towels to remove all residual water drops.
9. Dispense 100 µl of TMB Reagent solution into each well. Gently mix for 5 seconds.
10. Incubate at room temperature for 20 minutes.
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 15 minutes.

Calculation of Results

1. Calculate the average absorbance values (A450) for each set of reference standards, control, and samples.
2. Construct a standard curve by plotting the mean absorbance obtained for each reference standard against its concentration in ng/ml on linear graph paper, with absorbance on the vertical (y) axis and concentration on the horizontal (x) axis.
3. Using the mean absorbance value for each sample, determine the corresponding concentration of CK-MB in ng/ml from the standard curve.
4. Any values obtained for diluted samples must be further converted by applying the appropriate dilution factor in the calculation.

Example of a Standard Curve

Standards	Myoglobin (ng/ml)	OD450
STD1	25	0.191
STD2	100	0.628
STD3	250	1.445
STD4	500	2.178
STD5	1000	2.896

Sensitivity

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

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