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CALBIOTECH

Myoglobin ELISA

Catalog No.: MG017C (96 Tests)

INTENDED USE

The Calbiotech Inc. Myoglobin ELISA Kit is intended for the quantitative determination of Myoglobin in human serum or plasma. **For Research Use Only. For professional use only. Not for use in diagnostic procedures.**

SUMMARY AND EXPLANATION

Myoglobin, a heme protein with a molecular weight of approximately 17,500 Daltons is found in both cardiac and skeletal muscle. Myoglobin is an oxygen-binding protein in muscle tissue, released into the bloodstream following muscle injury. Research applications include studying muscle trauma, ischemia–reperfusion injury, and biomarker kinetics in experimental models.

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
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 NOT PROVIDED

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

Potential biohazardous materials:

1. The standards 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. Do not pipette by mouth. Do not smoke, eat, or drink in the areas in which specimens or kit reagents are handled.
3. The components in this kit are intended for use as an integral unit. The components of different lots should not be mixed.
4. It is recommended that standards, control and serum samples be run in duplicate
5. Optimal results will be obtained by strict adherence to this protocol. Accurate and precise pipetting, as well as following the exact time and temperature requirements prescribed are essential. Any deviation from this may yield invalid data.

REAGENT PREPARATION

This assay is designed for use with human serum or plasma samples obtained in compliance with applicable laws, regulations, and institutional policies. Handle and store samples using procedures appropriate for research use. Samples may be stored refrigerated (2–8 °C) for up to seven days, or frozen (–20 °C or below) for up to six months. Avoid repetitive freeze–thaw cycles.

1. All reagents should be brought to room temperature (20-25°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.
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 (20-25°C).

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 (20-25°C) for 45 minutes.
7. Remove the incubation mixture by flicking plate contents into a waste container.
8. Remove liquid from all wells. Wash wells three times with 300 µL of 1X wash buffer. Blot on absorbance paper or paper towel.
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.

STANDARD CURVE

CONCENTRATION (NG/ML)	O.D. 450 nm
0	0.045
25	0.191
100	0.628
250	1.445
500	2.178
1000	2.896