

REFERENCES

1. Kagen, L.J.: Myoglobin: Methods and Diagnostic Uses, *CRC Crit. Rev. Clin. Lab. Sci.*, 2:273; (1978).
2. Juronen, E.L., Viikmaa, M.H., and Mikelsarr, A.V.N.: Rapid, simple and sensitive antigen capture ELISA for the quantitation of myoglobin using monoclonal antibodies, *J. Imm. Meth.*: 111, 109; (1988).
3. Chapelle JP. et al.: Serum myoglobin determinations in the assessment of acute myocardial infarction. *Eur. Heart Journal*, 3:122, (1982).
4. Cairns, J.P., et.al.: Usefulness of serial determinations of myoglobin and creatine kinase in serum compared for assessment of acute myocardial infarction, *Clin. Chem. News*, 29: 469, (1983).
5. Silva, D.P., et.al.: Development and application of antibodies to human cardiac myoglobin in rapid fluorescence immunoassay, *Clin. Chem.*, 37: 1356, (1991).
6. Ellis AK.: Patters of myoglobin release after reperfusion of injured myocardium. *Clin. Chem.*, 72:639, (1985).
7. Mair J. et al.: Rapid diagnosis of myocardial infarction by immunoturbidimetric myoglobin measurement (letter). *Lancet*, ;337:1343, (1991).
8. Chapelle, J.P.: Myoglobin. *Clin. Chem. News*, 17:22, (1991).
9. Hamfelt, A., et. al.: Use of biochemical tests for myocardial infarction in the county of Vasternorrland, a clinical chemistry routine for the diagnosis of myocardial infarction. *Scand. J. Clin. Lab. Invest. Suppl.*, 200:20, (1990).
10. Tucker, J.F., et.al.: Early diagnostic efficiency of cardiac troponin I and Troponin T for acute myocardial infarction, *Academic Emergency Medicine*: 4(1): 13-21; (1997).
11. de Winter, R.J., et.al.: Value of myoglobin, troponin T and CK-Mbmass in ruling out an acute myocardial infarction in the emergency room, *Circulation*: 92(12): 3401-7; (1995).
12. Montague, C., Kircher, T.: Myoglobin in early evaluation of acute chest pain, *Amer. J. Clin. Path.*: 104(4): 472-6; (1995).
13. Tucker, J.F., et.al., Value of serial myoglobin levels in the early diagnosis of patients admitted for acute myocardial infarction, *Annals of Emergency Medicine*: 24(4): 704-8; (1994).
14. Roxin, L.E., et.al.: The value of serum myoglobin determinations in the early diagnosis of acute myocardial infarction, *Acta Medica Scand.*: 215(5): 417-25; (1984).
15. Sylven, C., Bendz, R.: Myoglobin, creatine kinase and its isoenzyme MB in serum after acute myocardial infarction, *Eur. J. Cardiol.*: 8(4-5): 515-21; (1978).
16. Norregaard-Hansen, K., et. al.: Early observations of S-myoglobin in the diagnosis of acute myocardial infarction. The influence of discrimination limit, analytical quality, patient's sex, and prevalence of disease. *Scand. J. Clin. Lab. Invest.*, 46:561-569, (1986).
17. Engvall, E., "Methods in Enzymology", Volume 70, VanVunakis H. and Langone, J.J. (eds.), Academic Press, New York, NY, 419-492, (1980).
18. Uotila, M., Ruuslahti, E. And Engvall, E., *J. Immunol. Methods*, 42, 11-15, (1981).
19. U.S. Department of Labor, Occupational Safety and Health Administration, 29 CFR Part 1910.1030. Occupational Exposure of Bloodborne Pathogens; Final Rule. Federal Register; 56(235):64175, (1991).
20. USA Center for Disease Control/National Institute of Health Manual, "Biosafety in Microbiological and Biomedical Laboratories", (1984).
21. National Committee for Clinical Laboratory Standards. Protection of Laboratory Workers from Instrument Biohazards and Infectious Disease Transmitted by Blood, Body Fluids, and Tissue: Approved Guideline. NCCLS Document M29-A, (1997).
22. Clinical Guide to Laboratory Tests. N.W. Tietz, Ed., 3rd Edition, W.B. Saunders, Co., p. 482, (1995).

2010-02-23

For Research Use Only. Not for use in Diagnostic Procedures.

Cat#: MG017C (96 Tests)

For Order and Inquiries, please contact

 Calbiotech Inc.,
10461 Austin Dr, Spring Valley, CA, 91978
Tel (619) 660-6162, Fax (619) 660-6970,
www.calbiotech.com



CEpartner4U, 3951DB; 13. NL.
tel: +31 (0)6.516.536.26



Myoglobin ELISA

Catalog No.: MG017C (96 Tests)

INTENDED USE

The Calbiotech Inc. (CBI) Myoglobin ELISA Kit is intended for the quantitative determination of Myoglobin in human serum or plasma.

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
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

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.
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

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. 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