Signosis
BioSignal Capture

Myoglobin ELISA

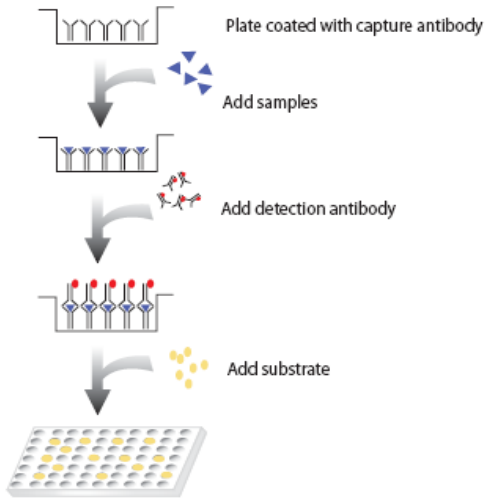
Catalog Number EA-0302 **(For Research Use Only)**

Introduction

Myoglobin, a heme protein, is found in both cardiac and skeletal muscle. Myoglobin holds oxygen inside heart and skeletal muscle. When muscle is damaged, as in a heart attack, larger amounts of myoglobin are released and blood levels rise rapidly (1-2). 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 (1-5). 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 (4, 6-7).

Principle of the assay

The myoglobin ELISA is based on the principle of a solid phase enzyme-linked immunosorbent assay. The assay utilizes a mouse monoclonal antibody against distinct determinants on myoglobin for immobilization on the microtiter wells and a goat anti-myoglobin antibody conjugated to horseradish peroxidase (HRP) for detection. The test sample is allowed to react simultaneously with these antibodies, resulting in myoglobin being sandwiched between the solid phase and enzyme-linked antibodies. After incubation, the wells are washed to remove unbound-labeled antibodies. A HRP substrate, TMB, is added to result in the development of a blue color. The color development is then 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.



ELISA Diagram

Materials provided with the kit

- Mouse monoclonal anti-myoglobin antibody-coated plate with 96 wells
- Reference Standard Set (1.0 ml/vial, 1 set/kit) Contains 0, 25, 100, 250, 500, and 1000 ng/ml myoglobin. These standards have been pre-diluted 10-fold, ready-to-use.
- Sample Diluent (25 ml/bottle) Contains bovine serum and 1.0% (w/v) Pro-Clin as preservative.
- Enzyme Conjugate Reagent, 22 ml
- TMB Reagent (one step), 11 ml
- Stop Solution (1N HCl), 11 ml.

Material required but not provided

- Microplate reader capable of measuring absorbance at 450 nm
- Deionized or distilled water.

Warning and precautions

1. Caution: This kit contains human material. The source material used for manufacture of this component tested negative for HBsAg, HIV 1/2 and HCV by FDA-approved methods. However, no method can completely assure absence of these agents. Therefore, all human blood products, including serum samples, should be considered potentially infectious. It is recommended that the reagents and patient samples be handled according to the OSHA Standard on Bloodborne Pathogens (8) or other appropriate national biohazard safety guidelines or regulations (9-11).
2. Avoid contact with 1N HCl. It may cause skin irritation and burns. If contact occurs, wash with copious amounts of water and seek medical attention if irritation persists.

Reagent preparation

All reagents should be allowed to reach room temperature (18-25°C) before use.
Reconstitute each lyophilized standard with 1.0 ml distilled water. Allow the reconstituted material to stand for at least 20 minutes and mix gently. The Reconstituted standards will be stable for up to 8 hours when stored sealed at 2-8 °C.
Discard the reconstituted Standards after 8 hours. To assure maximum stability of the reconstituted Standards, they should be aliquoted and frozen (–20°C or below) immediately after reconstitution has been achieved. Each aliquoted Standard should be frozen and thawed only once.

Assay procedure

1. Patient serum and control serum should be diluted 10 folds with Sample Diluent before use.
2. Add 20µl of myoglobin standards, diluted specimens and diluted controls into the appropriate wells.
3. Add 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. Rinse and flick the microwells 5 times with distilled or deionized water. (Please do not use tap water.)
8. Strike the wells sharply onto absorbent paper or paper towels to remove all residual water drops.
9. Add 100µl 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 100µl 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.

References

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Example of 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