

Product Information

Myoglobin ELISA

Catalog Number **SE120097**
Storage Temperature 2–8 °C

TECHNICAL BULLETIN

Product Description

Myoglobin, a heme protein with a molecular mass of ~17.5 kDa, 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 the 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 (~19 hours), myoglobin levels can be expected to peak within 6–9 hours.

The Myoglobin ELISA kit 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.

The Myoglobin ELISA kit is intended for the quantitative determination of myoglobin in human serum or plasma. It 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 microplate 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 (Tetramethylbenzidine) 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
Microwell coated with murine monoclonal anti-myoglobin.	12 x 8 x 1
Reference Standard Set	0.5 mL
Sample Diluent	25 mL
Enzyme Conjugate Reagent	22 mL
TMB Reagent	11 mL
Stop Solution	11 mL
Wash Concentrate 20x: 1 Bottle	25 mL

Reagents 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 450 nm
5. Absorbent paper or paper towel
6. Graph paper

Precautions and Disclaimer

This product is for R&D use only, not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Preparation Instructions20x Wash Buffer Concentrate

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).

Storage/Stability

Store the kit at 2–8 °C.

Procedure

Notes: The components in this kit are intended for use as an integral unit. The components of different lots should not be mixed.

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.

All reagents should be brought to room temperature (18–26 °C) before use.

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) of Sample Diluent.
Note: **Do not dilute** the standards – they have already been prediluted 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 (18–26 °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 absorbent 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 microplate well reader within 15 minutes.

ResultsStandard 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

References

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