ab108652 – Myoglobin Human ELISA Kit

Instructions for Use

An immunoenzymatic assay for the quantitative measurement of Myoglobin in Human Serum.

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

Version 2 Last Updated 1 February 2019

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1. BACKGROUND

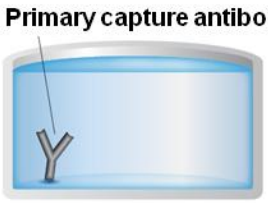
Abcam's Myoglobin *in vitro* ELISA (Enzyme-Linked Immunosorbent Assay) kit is designed for the accurate quantitative measurement of Myoglobin in Human serum.

A 96-well plate has been precoated with anti-Myoglobin antibodies. Samples and standards and are added to the wells, where Myoglobin in the sample and standards binds to the precoated antibody. Added anti-Myoglobin HRP conjugate binds to this antibody-Myoglobin complex. After incubation, the wells are washed to remove unbound material and TMB substrate is then added which is catalyzed by HRP to produce blue coloration. The reaction is terminated by addition of Stop Solution which stops the color development and produces a color change from blue to yellow. The intensity of signal is directly proportional to the amount of Myoglobin in the sample and the intensity is measured at 450 nm.

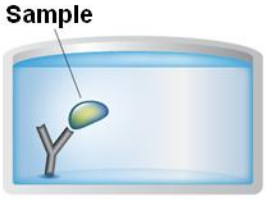
Myoglobin, a heme protein with a molecular weight of approximately 17,500 Daltons is found in both cardiac and skeletal muscle. Damage to either type of muscle following conditions such as trauma, ischemia, and diseases that cause myopathy, is associated with the release of myoglobin into serum. Specifically, 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. 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.

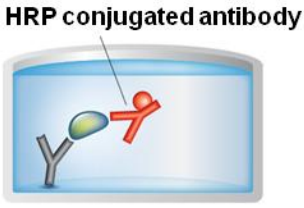
2. ASSAY SUMMARY



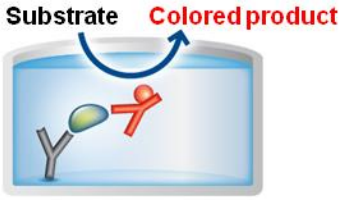
Prepare all reagents, samples and standards as instructed.



Add samples and standards to wells used.



Add prepared labeled HRP-Conjugate to each well. Mix well. Incubate at room temperature.



After washing, add TMB substrate solution to each well. Incubate at room temperature. Add Stop Solution to each well. Read immediately.

3. PRECAUTIONS

Please read these instructions carefully prior to beginning the assay.

All kit components have been formulated and quality control tested to function successfully as a kit. Modifications to the kit components or procedures may result in loss of performance.

4. STORAGE AND STABILITY

Store kit at 2-8°C immediately upon receipt.

Refer to list of materials supplied for storage conditions of individual components. Observe the storage conditions for individual prepared components in section 9. Reagent Preparation.

5. MATERIALS SUPPLIED

Item	Amount	Storage Condition (Before Preparation)
Anti-Myoglobin Coated Microplate (12 x 8 wells)	96 Wells	2-8°C
Stop Solution	11 mL	2-8°C
Anti-Myoglobin HRP Conjugate	22 mL	2-8°C
Sample Diluent	25 mL	2-8°C
TMB Substrate Solution	11 mL	2-8°C
Myoglobin Standard 0 – 0 ng/mL	0.5 mL	2-8°C
Myoglobin Standard 1 – 25 ng/mL	0.5 mL	2-8°C
Myoglobin Standard 2 – 100 ng/mL	0.5 mL	2-8°C
Myoglobin Standard 3 – 250 ng/mL	0.5 mL	2-8°C
Myoglobin Standard 4 – 500 ng/mL	0.5 mL	2-8°C
Myoglobin Standard 5 – 1,000 ng/mL	0.5 mL	2-8°C

6. MATERIALS REQUIRED, NOT SUPPLIED

These materials are not included in the kit, but will be required to successfully utilize this assay:

- Microplate reader capable of measuring absorbance at 450 nm or 620 nm
- Multi- and single-channel pipettes to deliver volumes between 10 and 1,000 µL
- Optional: Automatic plate washer for rinsing wells.
- Rotating mixer
- Deionised or (freshly) distilled water.
- Disposable tubes
- Timer
- Absorbent paper or paper towel.

7. LIMITATIONS

- ELISA kit intended for research use only. Not for use in diagnostic procedures
- All components of Human origin used for the production of these reagents have been tested for anti-HIV antibodies, anti-HCV antibodies and HBsAg and have been found to be non-reactive. Nevertheless, all materials should still be regarded and handled as potentially infectious
- Use only clean pipette tips, dispensers, and lab ware
- Do not interchange screw caps of reagent vials to avoid cross-contamination
- Close reagent vials tightly immediately after use to avoid evaporation and microbial contamination
- After first opening and subsequent storage check conjugate and control vials for microbial contamination prior to further use
- To avoid cross-contamination and falsely elevated results pipette patient samples and dispense conjugate, without splashing, accurately to the bottom of wells
- Serum samples demonstrating gross lipemia, gross hemolysis, or turbidity should not be used with this test.
- Patient samples may contain human anti-mouse antibodies (HAMA) which are capable of giving falsely elevated results with assays that utilize mouse monoclonal antibodies. The Myoglobin ELISA assay has been designed to minimize interference from HAMA-containing specimens; nevertheless complete elimination of this interference from all patient specimens cannot be guaranteed.

8. TECHNICAL HINTS

- Avoid foaming or bubbles when mixing or reconstituting components
- Avoid cross contamination of samples or reagents by changing tips between sample, standard and reagent additions
- Ensure plates are properly sealed or covered during incubation steps
- Complete removal of all solutions and buffers during wash steps is necessary for accurate measurement readings
- Addition of the TMB Substrate solution initiates a kinetic reaction, which is terminated by the addition of the Stop Solution. Therefore, the TMB Substrate and the Stop Solution should be added in the same sequence to eliminate any time deviation during the reaction
- It is important that the time of reaction in each well is held constant for reproducible results. Pipetting of samples should not extend beyond ten minutes to avoid assay drift. If more than 10 minutes are needed, follow the same order of dispensation. If more than one plate is used, it is recommended to repeat the dose response curve in each plate
- Good laboratory practice requires that controls are run with each calibration curve. A statistically significant number of controls should be assayed to establish mean values and acceptable ranges to assure proper performance.
- The incomplete or inaccurate liquid removal from the wells could influence the assay precision and/or increase the background
- **This kit is sold based on number of tests. A 'test' simply refers to a single assay well. The number of wells that contain sample, control or standard will vary by product. Review the protocol completely to confirm this kit meets your requirements. Please contact our Technical Support staff with any questions**

9. REAGENT PREPARATION

Equilibrate all reagents, samples and controls to room temperature (18-25°C) prior to use.

- Do not dilute the standards – they have already been pre-diluted 10-fold.
- All other solutions are supplied ready to use

10. SAMPLE COLLECTION AND STORAGE

- The determination of Myoglobin can be performed in Human serum. Specimens should be collected using standard venipuncture techniques. Remove serum from the coagulated or packed cells within 60 minutes after collection. Specimens which cannot be assayed within 24 hours of collection should be frozen at -20°C or lower, and will be stable for up to six months. Specimens should not be repeatedly frozen and thawed prior to testing. DO NOT store in “frost free” freezers, which may cause occasional thawing. Specimens which have been frozen, and those which are turbid and/or contain particulate matter, must be centrifuged prior to use.

  Avoid repeated freezing and thawing

- 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.
- Samples with expected myoglobin concentrations over 1,000 ng/mL may be quantitated by further dilution 10-fold with sample diluent.

11. PLATE PREPARATION

- The 96 well plate strips included with this kit are supplied ready to use. It is not necessary to rinse the plate prior to adding reagents
- Unused well strips should be returned to the plate packet and stored at 4°C
- For each assay performed, a minimum of 1 well must be used as a blank, omitting sample and conjugate from well addition
- For statistical reasons, we recommend each standard and sample should be assayed with a minimum of two replicates (duplicates)

12. ASSAY PROCEDURE

- **Equilibrate all materials and prepared reagents to room temperature prior to use.**
- **Please read the test protocol carefully before performing the assay. Result reliability depends on strict adherence to the test protocol as described.**
- **If performing the test on ELISA automatic systems we recommend increasing the washing steps from three to five and the volume of washing solution from 300 µL to 350 µL to avoid washing effects.**
- **Assay all standards, controls and samples in duplicate.**

13.1. Prepare all reagents, working standards, and samples as directed in the previous sections.

13.2. Remove excess microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, reseal and return to 4°C storage.

13.3. Add 20 µL standards, control and samples into their respective wells.

13.4. Add 200 µL of anti-Myoglobin-HRP conjugate into each well. Mix gently for 30 seconds.

Note: Complete mixing is essential for good assay performance

13.5. Cover wells with the foil supplied in the kit and incubate at room temperature for 45 minutes.

13.6. Remove the foil, aspirate the contents of the wells and wash each well five times with 300 µL of deionized or distilled water. Avoid spill over into neighboring wells. The soak time between each wash cycle should be >5 sec. After the last wash, remove the remaining deionized or distilled water by aspiration or decanting. Invert the plate and blot it against clean paper towels to remove excess liquid.

Note: Complete removal of liquid at each step is essential for good assay performance.

13.7. Add 100 µL TMB Reagent into each well. Mix gently for 5 seconds.

13.8. Incubate at room temperature for 20 minutes in the dark.

13.9. Stop the reaction by adding 100 µL of Stop Solution to each well. Mix gently for 30 seconds.

Note: It is important to make sure that all the blue color changes to yellow color completely.

13.10. Measure the absorbance of the sample at 450 nm within 15 minutes of addition of the Stop Solution.

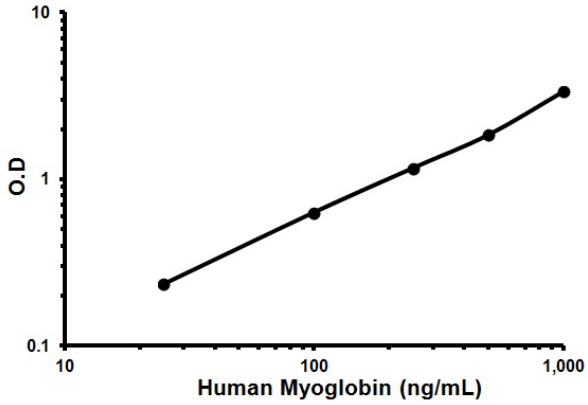
13. CALCULATIONS

Calculate the mean background subtracted absorbance for each point of the standard curve and each sample. Plot the mean value of absorbance of the standards against concentration. Draw the best-fit curve through the plotted points. (e. g.: Four Parameter Logistic).

Interpolate the values of the samples on the standard curve to obtain the corresponding values of the concentrations expressed in pg/mL.

14. TYPICAL DATA

TYPICAL STANDARD CURVE – Data provided for **demonstration purposes only**. A new standard curve must be generated for each assay performed.



Conc. (ng/mL)	Mean O.D. (-Blank)
0	0.071
25	0.235
100	0.632
250	1.169
500	1.845
1,000	3.357

15. TYPICAL SAMPLE VALUES

EXPECTED NORMAL VALUES –

Normal serum Myoglobin levels range from 12 to 100 ng/mL. Values increase slightly with age.

Using the Myoglobin Human ELISA kit, an evaluation of the clinical data was conducted to determine the normal expected value of the kit. The study yielded normal range values in agreement with industry standards. Eighty-three apparently healthy adults were assayed using the test to establish the normal expected value. The range was found to be between 8.1 and 54.5 ng/mL myoglobin.

Each facility should establish its own reference intervals for myoglobin as performed on ELISA test. Other factors should also be considered in the diagnosis of myocardial infarction, as any condition resulting in skeletal or cardiac muscle damage may potentially increase myoglobin levels above the expected normal range.

Note: Serial sampling may be required to detect elevated levels.

SENSITIVITY –

The minimum detectable concentration of the Myoglobin ELISA assay is estimated to be 5 ng/mL.

HOOK EFFECT –

No high-dose hook effect is observed in this test with patient sample concentrations up to 10,000 ng/mL.

PRECISION –

Number of replicates	Myoglobin (ng/mL)	S.D.	Intra-Assay %CV
20	55.6	2.2	3.9
20	214.3	12.9	6.0
20	294.9	16.2	5.5
20	505.9	26.3	5.2
20	1,437	94.0	6.6

Number of replicates	Myoglobin (ng/mL)	S.D.	Inter-Assay %CV
35	59.2	4.6	7.8
35	244.4	12.8	5.2
35	330.5	38.9	11.8
35	568.3	52.7	9.3
35	1,451.7	104.7	7.2

LINEARITY –

Three patient samples were serially diluted to determine linearity. The mean recovery was 105.8%.

RECOVERY –

Various patient serum samples of known myoglobin levels were combined and assayed in duplicate. The mean recovery was 102.8%.

Pair No.	Expected [Myoglobin] (ng/mL)	Observed [Myoglobin] (ng/mL)	% Recovery
1	280	250	89.3
2	451	495	109.8
3	255	241	94.5
4	269	300	111.5
5	39	41	105.1
6	240	231	96.0
7	92	88	95.9
8	209	214	102.0
9	340	328	96.0
10	214	213	100.0
11	551	655	118.8
12	431	436	101.2
13	757	824	108.8
14	747	768	102.8
15	780	894	114.6

16. ASSAY SPECIFICITY

CROSS REACTIVITY –

This kit detects Myoglobin in Human samples. There is no cross-reactivity with related cardiac or skeletal enzymes.

Other species have not yet been tested with this kit.

17. TROUBLESHOOTING

Problem	Cause	Solution
Low signal	Incubation time to short	Try overnight incubation at 4 °C
	Precipitate can form in wells upon substrate addition when concentration of target is too high	Increase dilution factor of sample
	Using incompatible sample type (e.g. serum vs. cell extract)	Detection may be reduced or absent in untested sample types
	Sample prepared incorrectly	Ensure proper sample preparation/dilution
Large CV	Bubbles in wells	Ensure no bubbles present prior to reading plate
	All wells not washed equally/thoroughly	Check that all ports of plate washer are unobstructed/wash wells as recommended
	Incomplete reagent mixing	Ensure all reagents/master mixes are mixed thoroughly
	Inconsistent pipetting	Use calibrated pipettes & ensure accurate pipetting
	Inconsistent sample preparation or storage	Ensure consistent sample preparation and optimal sample storage conditions (e.g. minimize freeze/thaws cycles)

Problem	Cause	Solution
High background	Wells are insufficiently washed	Wash wells as per protocol recommendations
	Contaminated wash buffer	Make fresh wash buffer
	Waiting too long to read plate after adding stop solution	Read plate immediately after adding stop solution
Low sensitivity	Improper storage of ELISA kit	Store all reagents as recommended. Please note all reagents may not have identical storage requirements.
	Using incompatible sample type (e.g. Serum vs. cell extract)	Detection may be reduced or absent in untested sample types

18. NOTES

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