DRG® Myoglobin (EIA-3955)

Revised 29 July 2010 rm (Vers. 4.1) **IVD**

Please use only the valid version of the package insert provided with the kit.

For In Vitro Diagnostic Use Only
Store at 2 to 8°C.

Proprietary and Common Names
Human Myoglobin Enzyme Immunoassay

Intended Use

The Myoglobin ELISA is intended for the quantitative determination of myoglobin in human serum.

Introduction

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.[1,2] 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.[1,3,4,5]
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] A number of reports suggest using the measurement of myoglobin as a diagnostic aid in ruling out myocardial infarction[5,8] 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 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.[16]

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.[17,18] 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.

Reagents

MATERIALS PROVIDED WITH THE KIT

Antibody-Coated Wells (1 plate, 96 wells)
Microtiter wells coated with murine monoclonal anti-myoglobin.

Reference Standard Set (0.5 ml/vial, 1 set/kit)
Contains 0, 25, 100, 250, 500, and 1000 ng/ml myoglobin, liquid, ready-to-use.
These standards have been pre-diluted 10-fold. Please do not dilute them again.

Sample Diluent (25 ml/bottle)
Contains phosphate buffer and 1.0% (w/v) Pro-Clin as preservative.

Enzyme Conjugate Reagent (22 ml/vial)
Contains anti-myoglobin conjugated to horseradish peroxidase in Tris buffer-BSA solution with preservatives.

TMB Reagent (11 ml/bottle)
Contains one-step TMB solution.

Stop Solution (1 bottle, 11 ml/bottle)
Contains diluted hydrochloric acid (1N HCl).

MATERIALS REQUIRED BUT NOT PROVIDED

- Precision pipettes: 20 µl , 50 µl, 200 µl, and 1.0 ml
- Disposable pipette tips
- Distilled water
- Vortex mixer or equivalent.
- Absorbent paper or paper towels
- Graph paper
- Microtiter plate reader

Warnings and Precautions for Users

1. CAUTION: This kit contains human material. The source material used for manufacture of this kit 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. Handling and disposal should be as defined by an appropriate national biohazard safety guideline or regulation, where it exists.21
2. Do not use reagents after expiration date and do not mix or use components from kits with different lot numbers.
3. Do not use the reagent when it becomes cloudy or contamination is suspected.
4. Do not use the reagent if the vial is damaged.
5. Replace caps on reagents immediately. Do not switch caps.
6. Each well can be used only once.
7. Do not pipette reagents by mouth.
8. Solutions containing additives or preservatives, such as sodium azide, should not be used in the enzyme reaction.
9. 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.
10. For in vitro diagnostic use.

Storage Conditions

1. Store the unopened kit at 2-8°C upon receipt and when it is not in use, until the expiration shown on the kit label. Refer to the package label for the expiration date.
2. The opened and used reagents are stable until the expiration date if stored properly at 2-8°C.
3. Keep microtiter plate in a sealed bag with desiccant to minimize exposure to damp air.

Instrumentation

A microtiter well reader with a bandwidth of 10 nm or less and an optical density range of 0 to 3 OD or greater at 450 nm wavelength is acceptable for absorbance measurement.

Reagent Preparation

All reagents should be brought to room temperature (18-25°C) before use.

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.

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PLEASE DO NOT DILUTE THE STANDARDS – THEY HAVE ALREADY BEEN PRE-DILUTED 10-FOLD.

Samples with expected myoglobin concentrations over 1000 ng/ml may be quantitated by further dilution 10-fold with sample diluent.

Specimen Collection and Preparation

The use of SERUM samples is required for this test.
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.

Procedural Notes

- Pipetting Recommendations (single and multi-channel): Pipetting of all standards, samples, and controls should be completed within 3 minutes.
- All standards, samples, and controls should be run in duplicate concurrently so that all conditions of testing are the same.
- It is recommended that the wells be read within 15 minutes following addition of Stop Solution.

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. Rinse and flick the microwells 5 times with distilled or deionized water. (Please do not use tap water.)
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**.

Quality Control

Good laboratory practice requires that quality control specimens (controls) be run with each calibration curve to verify assay performance.
To ensure proper performance, control material should be assayed repeatedly to establish mean values and acceptable ranges.

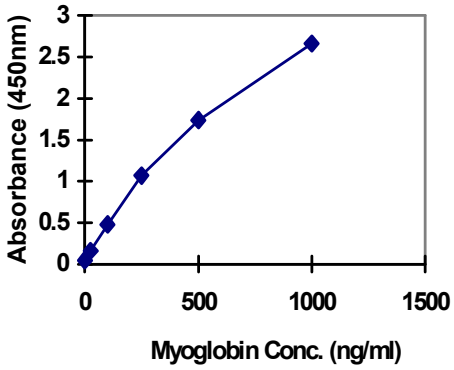
Calculation of Results

1. Calculate the mean absorbance value (A450) for each set of reference standards, controls and samples.
2. Construct a standard curve by plotting the mean absorbance obtained for each reference standard against its concentration in ng/ml on graph paper, with absorbance values on the vertical or Y axis, and concentrations on the horizontal or X axis.
3. Use the mean absorbance values for each specimen to determine the corresponding concentration of myoglobin in ng/ml from the standard curve.
4. Since the reference standards have already been pre-diluted 10-fold, there is no need for the patient samples or control sera observed values to be multiplied by the dilution factor of 10. However, if the patient samples are diluted to 100-fold, the observed values should be multiplied by 10.

Example of Standard Curve

Results of a typical standard run with optical density readings at 450 nm shown in the Y axis against myoglobin concentrations shown in the X axis. This standard curve is for illustrative purpose only, and should not be used to calculate unknowns. Each laboratory should obtain its own data and standard curve.

Myoglobin (ng/ml)	Absorbance (450 nm)
0	0.046
25	0.158
100	0.476
250	1.070
500	1.741
1000	2.664



Limitations of the Procedure

Reliable and reproducible results will be obtained when the assay procedure is carried out with a complete understanding of the package insert instructions and with adherence to good laboratory practice.
Diagnostic results obtained from the Myoglobin ELISA should be used in conjuction with other diagnostic procedures and information available to the physician.; e.g., additional clinical testing, ECG, symptoms, and clinical observations.
The wash procedure is critical. Insufficient washing will result in poor precision and falsely elevated absorbance readings.
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.
Test results that are inconsistent with the clinical picture and patient history should be interpreted with caution.

Expected Normal Values

Normal serum myoglobin levels range from 12 to 100 ng/ml. Values increase slightly with age.[22]
Using the Myoglobin ELISA, 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 (83) 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.

Performance Characteristics

CLINICAL PERFORMANCE

A clinical investigation was conducted to determine the accuracy of the Myoglobin ELISA as compared to the Abbott AxSym Myoglobin MEIA. The data is presented below.
A statistical study using 150 clinical patient serum samples, ranging in myoglobin concentration from 3.7 ng/ml to 919.8 ng/ml as analyzed using the Myoglobin ELISA (EIA-3955) (13.0 ng/ml to 1011.0 ng/ml Abbott Myoglobin MEIA), demonstrated equivalent correlation with the AxSym Myoglobin kit as shown below.

Correlation coefficient = 0.9392
Slope = 0.8871
Intercept = 55.051
EIA-3955 Mean = 287.9 ng/ml
Abbott Myoglobin Mean = 262.5 ng/ml

SENSITIVITY

The lowest detectable level of myoglobin by this 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

a. Intra-Assay Precision

Within-run precision was determined by replicate determinations of five different serum samples in one assay. Within-assay variability is shown below:

Serum Sample	1	2	3	4	5
# Reps.	20	20	20	20	20
Mean Myo. (ng/ml)	55.6	214.3	294.9	505.9	1,437
S.D.	2.2	12.9	16.2	26.3	94.0
C.V. (%)	3.9%	6.0%	5.5%	5.2%	6.6%

b. Inter-Assay Precision

Between-run precision was determined by replicate measurements of five different serum samples over a series of individually calibrated assays. Between-assay variability is shown below:

Serum Sample	1	2	3	4	5
# Replicates	35	35	35	35	35
Mean Myo (ng/ml)	59.2	244.4	330.5	568.3	1451.7
S.D.	4.6	12.8	38.9	52.7	104.7
C.V. (%)	7.8%	5.2%	11.8%	9.3%	7.2%

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	575	569	*98.9%*

LINEARITY

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

#	Dilution	Expected Conc. (ng/ml)	Observed Conc. (ng/ml)	% Expected
1.	Undiluted	-----	-----	-----
	1:2	540	542.6	100.5%
	1:4	270	290.8	107.7%
	1:8	135	153.3	113.6%
	1:16	67.5	75.3	111.6%
	1:32	33.8	38.7	114.5%
	1:64	16.9	18.8	111.2%
	1:128	8.5	8.6	101.2%
	1:256	4.3	3.9	90.7%
	Mean = 106.4%			
2.	Undiluted	-----	-----	-----
	1:2	945	956	101.2%
	1:4	472.5	500	105.8%
	1:8	236.3	262.8	111.2%
	1:16	118.1	131.7	111.5%
	1:32	59.1	65.2	110.3%
	1:64	29.5	31.1	105.4%
	1:128	14.8	12.8	86.5%
	Mean =104.6 %			
3.	Undiluted	-----	-----	-----
	1:2	-----	-----	-----
	1:4	691.0	691.4	100.0%
	1:8	362.3	345.7	104.8%
	1:16	173.9	172.8	100.6%
	1:32	95.7	86.4	110.8%
	1:64	45.8	43.2	106.0%
	1:128	21.2	21.6	98.0%
	1:256	13.5	10.8	125.0%
	Mean = 106.5%			

Specificity

The following materials were tested for cross-reactivity at concentrations up to the levels indicated below. No cross-reactivity was observed for any of the components.MATERIAL TESTED	TEST CONCENTRATION
Interfering Substances	
Cardiac Tnl	1000 ng/ml
Cardiac TnT	1000 ng/ml
Cardiac TnC	1000 ng/ml
Skeletal Tnl	1000 ng/ml
CK-MB	1000 ng/ml
CK-MB2	100 ng/ml
CK-MM	5 µg/ml
CK-BB	10 µg/ml
Tropomyosin	1000 ng/ml
Myosin Light Chain Kinase (MLCK)	1000 ng/ml
Actin	1000 ng/ml
Endogenous Substances	
Billirubin	20 mg/dl
Cholesterol	500 mg/dl
Triglyceride	1,500 mg/dl
Total Protein	3 g/dl
Total Protein	10 g/dl
Therapeutic Substances	
Aspirin	0.3 ng/ml
Coumadin	1000 µg/ml
Digoxin	200 ng/ml
Flurosemide (Lasix)	400 µg/ml
Sodium Heparin	8 U/ml

Standardization

Human myoglobin Complex was obtained from a qualified vendor, and myoglobin concentration was determined. The material was further diluted with the Myoglobin Sample Diluent and served as "Standard Stock Solution" for

preparing myoglobin reference Standard Sets. The target value of the "Standard Stock Solution" was confirmed by the Abbott AxSym Myoglobin immunoassay.

References

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