



Myoglobin (CLIA) PACKAGE INSERT



INTENDED USE

Myoglobin (CLIA) is a chemiluminescence immunoassay test for the quantitative measurement of circulating Myoglobin (CLIA) in human serum, anticoagulated whole blood and plasma, which is intended as an aid to diagnosis of myocardial infarction. The assay can be used for samples over the range of 1ng/mL~1000ng/mL.

The test must be performed only on the ACCRE series analyzers (including ACCRE 6, ACCRE 8, ACCRE 90, ACCRE 100, ACCRE 120 and other ACCRE series analyzers).

For *in vitro* diagnostic use only. For professional use only.

SUMMARY

Myoglobin (MYO) is an intracellular protein mainly founded in striated muscles such as cardiac and skeletal muscle in humans and mammals. It is a polypeptide chain containing 153 amino acids and a heme prosthetic group with a molecular weight of 17.5 KD. It has the ability of reversibly oxygen-binding, so it could transport and store oxygen in muscle cells¹.

MYO is more sensitive than cardiac troponin I (cTnI) in the diagnosis of cardiac infarction. When cardiac cells are damaged, MYO is quickly released into the blood. MYO releases into the circulation as early as 2 hours after acute myocardial infarction (AMI), peaks between 4-12 hours, and returns to normal after 24 hours. Therefore, MYO has been used as an early indicator of myocardial infarction and a clinical monitor combined with CK-MB and cTnI. In addition, MYO also has abnormal increase in renal dysfunction and skeletal muscle injury, therefore its specificity is not high. MYO is more sensitive in early AMI than other cardiac markers, so it is an important indicator in the early diagnosis of AMI, early re-infarction, and observation of successful reperfusion after suppository treatment²⁻⁵.

ASSAY PRINCIPLES

The principle of Myoglobin (CLIA) hereinafter also referred to as the "MYO" combines a two-site immunoassay method with enzyme catalytic chemiluminescence immunoassay (CLIA). The principle is described as following:
Reagents for the assay are ready-to-use and pre-dispensed in the sealed reagent cartridges. All the assay steps are performed automatically by the ACCRE analyzers. The sample is manually pipetted into the sample well of reagent cartridge, then sample, Myoglobin antibody labeled with alkaline phosphatase and Myoglobin antibody coated magnetic particles are mixed thoroughly together, incubating at 37 °C. The antigen in the sample, labelled antibody and magnetic particles are bonded together as an antibody-antigen sandwich immuno-complex.

The immuno-complex is then washed in the magnetic field for 3 times to eliminate the unbound components in the sample. The substrate is added to trigger the chemiluminescent reaction, the result of chemiluminescent reaction is measured by photomultiplier tube (PMT) as relative light units (RLUs). The concentration of the substance to be detected in the sample is proportional to the luminescence intensity. At the end, the results are automatically calculated by the ACCRE analyzers in relation to the calibration curve stored.

CONTENT OF THE KIT

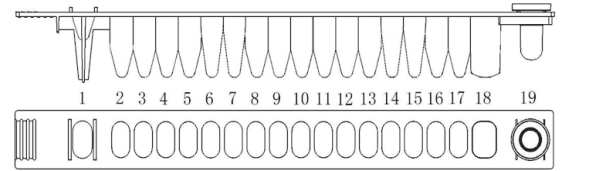
Package: 60 tests/kit with quality controls, 36 tests/kit with quality controls, 60 tests/kit without quality controls, 36 tests/kit without quality controls
The calibrator in the kit has been traced to Beckman Coulter myoglobin assay kit

(chemiluminescence) and its system. For specific calibrator concentration values, see the Calibrator Barcode Sheet for each lot.

Contents	Quantities				Notice
	REF W123A	REF W123C	REF W16A	REF W16C	
MYO Reagents Cartridges	60 strips	36 strips	60 strips	36 strips	Ready to Use
MYO Calibrator 1 (CAL1)	Dissolved to 1.0 mL x 1 vial	Dissolved to 1.0 mL x 1 vial	Dissolved to 1.0 mL x 1 vial	Dissolved to 1.0 mL x 1 vial	Lyophilized powder, Tris Buffer, 50mmM, Proclin300, 0.48g/L; BND, 0.2g/L
MYO Calibrator 2 (CAL2)	Dissolved to 1.0 mL x 1 vial	Dissolved to 1.0 mL x 1 vial	Dissolved to 1.0 mL x 1 vial	Dissolved to 1.0 mL x 1 vial	Lyophilized powder, human myoglobin protein, 25ng/mL
MYO Calibrator3 (CAL3)	Dissolved to 1.0 mL x 1 vial	Dissolved to 1.0 mL x 1 vial	Dissolved to 1.0 mL x 1 vial	Dissolved to 1.0 mL x 1 vial	Lyophilized powder, human myoglobin protein, 500ng/mL
MYO Quality Control Level 1 (QC1)	Dissolved to 1.0 mL x 1 vial	Dissolved to 1.0 mL x 1 vial	/	/	Lyophilized powder, human myoglobin protein, 25ng/mL Only for the configuration with Quality Controls
MYO Quality Control Level 2(QC2)	Dissolved to 1.0 mL x 1 vial	Dissolved to 1.0 mL x 1 vial	/	/	Lyophilized powder, human myoglobin protein, 500ng/mL Only for the configuration with Quality Controls
Instruction for Use	1	1	1	1	N/A
Reagent Registration Card	1	1	1	1	N/A
Calibrator Barcode Sheet	1	1	1	1	N/A
Quality Control Sheet	1	1	/	/	Only for the configurations with Quality Controls

The Reagents Cartridges

The strip consists of 19 wells. The 1st well is the sample well and the last 19th is the reading well for chemiluminescent signal. From the 2nd to the 18th well are all covered with a label, aluminum foil seal. The label comprises a barcode which mainly indicates the reagent assay information, reagent lot number and expiration date. All reagents components needed for the assay are ready-to-use and pre-dispensed in the cartridge. And please do not interchange integral component from different reagents or lots.



Description of the reagent cartridge

Well	Component	Content	Volume
3	Sample treatment solution (R3)	Tris buffer, 50mmol/L; ProClin300, 0.48g/L; BND, 0.2g/L	50 µL
5	Enzyme Label (R2)	ALP (alkaline phosphatase) labeled anti-MYO antibody (mouse), 2.0mg/L; MES Buffer, 50mmol/L; ProClin300, 0.48g/L; BND, 0.2g/L	50 µL
7	Magnetic Particles (R1)	Anti-MYO antibody (mouse) coated magnetic particles, 0.2g/L; Tris buffer, 50mmol/L; ProClin300, 0.48g/L; BND, 0.2g/L	50 µL
10, 11, 12, 14	Wash Solutions	Tris buffer, 50mmol/L; ProClin300, 0.48g/L; BND, 0.2g/L	350 µL
16	Substrate Solution	Disodium [(4-chlorophenyl)sulfonyl] (10-methyl-9(10H)-acridinylidene) methyl phosphate	200 µL

Note: Well 1, 2, 4, 6, 8, 9, 13, 15, 17, 18 are empty.

MATERIALS AND DISPOSABLES REQUIRED BUT NOT PROVIDED

- Sample diluent (manufactured by Tisenc).
- Pipette with disposable tip to dispense 200 µL.
- Powderless, disposable gloves.
- For other specific materials and disposables, please refer to the Instrument User Manual.
- Instruments of the ACCRE series analyzers.

WARNINGS AND PRECAUTIONS

- For *in vitro* diagnostic use only.
- For professional use only by qualified laboratory personnel.
- This kit contains products of animal origin. Certified knowledge of the origin and/or sanitary state of the animals does not totally guarantee the absence of transmissible pathogenic agents. It is therefore recommended that these products be treated as potentially infectious, and handled observing the usual safety precautions (do not ingest; do not inhale)
- The kit contains products of human origin.No known analysis method can totally guarantee the absence of transmissible pathogenic agents.It is therefore recommended that these products be treated as potentially infectious and handled observing the usual safety precautions (see Laboratory Biosafety Manual - WHO - Geneva - latest edition).
- Do not use reagents after the expiration date indicated on the box label.
- Do not use if the packaging of reagent cartridge is damaged or leaked.
- Do not use the reagents if the quantity is obviously insufficient.
- If any component adheres to inside wall of wells or aluminum film of the reagent cartridge, please gently switch the reagent cartridge to let the component back the wells.
- Do not reverse the reagent cartridge. Otherwise, the result will be unreliable.
- Do not mix reagents (or disposables) from different lots.
- Package insert instructions must be carefully followed. Reliability of assay results cannot be guaranteed if there are any deviations from the instructions in this package insert
- Use powderless gloves, as powder has been reported to cause false results for certain enzyme immunoassay tests.

STORAGE

- Store the Myoglobin (CLIA) kits including reagent cartridges, calibrators and quality controls upright at 2-8°C, kit can be stored in dark place and the kit can be valid for 18 months from manufactured date.
- After the reagent cartridge is on-board in the ACCRE analyzer, (Incubation temperature:37±0.5°C) the testing should be finished within 2 hours.

- When opened and re-dissolved, the calibrators and quality controls will be stable for 45 days at -20°C and below -20°C. Calibrators and quality controls can be frozen and thawed 3 times.
- Keep away from sunlight.
- Do not freeze reagent cartridges.

SAMPLES

The sample volume for Myoglobin (CLIA) assay is 50 µL, please note that at least 75 µ L of sample should be pipetted into the reagent cartridge.

Sample type and collection

Human serum or plasma/whole blood (Heparin lithium/EDTA-K₂) are recommended. Types of tubes:

- Plastic tube with clot activator,
- Plastic tube with clot activator and separation gel,
- Plastic tube with Heparin lithium,
- Plastic tube with EDTA -K₂.

It is recommended to validate collection tubes before using them as some may contain substances which interfere with test results.

Note: Blood sampling tube results may vary from one manufacturer to another depending on the materials and additives used.

It is the responsibility of each laboratory to validate the type of sample tube used and to follow the manufacturer's recommendations for use.

Sample preparation

The current revision of the WHO/DIL/LAB/99.1 document provides recommendations for the preparation of samples.
For use of the sample tubes, follow the tube manufacturer's instructions for use. The pre-analytical step, including the preparation of blood samples, is an essential part of medical analyses. In accordance with Good Laboratory Practice, this step is performed under the responsibility of the laboratory manager. Insufficient clot time can result in the formation of fibrin with micro-clots that are invisible to the naked eye. The presence of fibrin, red blood cells, or suspended particles can lead to erroneous results. Samples containing suspended fibrin particles or erythrocyte stroma should be centrifuged before testing. If there is a lipid layer over the sample after centrifuging, please transfer the clear sample into a new sample tube without transferring the lipid layer. Severe hemolysis samples (hemoglobin > 500 mg/dL) or heat-inactivated samples are not recommended for applying on the reagents. Preparation of frozen-stored samples: after thawing, these samples must be thoroughly mixed before testing. Mix using with a vortex-type mixer. If necessary, clarify samples by centrifuging before testing.

Sample stability

Serum and plasma samples can be stored at 15~30°C for up to 4 hours or at 2~8°C for up to 24 hours; if longer storage is required, freeze the serum or plasma at -20°C and below -20°C for up to 3 months, without exceeding 3 freeze/thaw cycles. Whole blood samples can be stored at 15~30°C for up to 4 hours or at 2~8°C for up to 24 hours and not undergo freeze/thaw cycles.

INSTRUCTIONS FOR USE

For complete instructions, see the Instrument User Manual.

When using the assay for the first time. Register the assay using the registration code in the reagent kit before any assay is used in the ACCRE analyzer for the first time. Calibration, QC and sample test are only available after assay was registered successfully.

When opening a new lot of reagents. Each new lot of reagent must be registered by using the registration code in Reagent Registration Card within the reagent kit.

Calibration

Calibration must be performed with the reagents and calibrators from Manufacturer. Each bottle of lyophilized calibrator should be re-dissolved by purified water to 1.0mL before use.

Calibration is necessary under the following conditions:

- (1) New reagent lot number applied;
- (2) Same lot of reagents used more than 4 weeks;
- (3) Quality control out of control;
- (4) When necessary, for example: system maintenance or repairment that may affect the analytical performance.

Test Procedure

1. Remove the kit from storage at 2-8°C and take out the required number of reagent cartridge. Allow the reagent cartridges, specimens, calibrators, and/or controls to equilibrate to room temperature (15-30°C) prior to testing.
2. Use each assay MYO cartridge for each sample, calibrator or control respectively.
3. Place the reagent cartridge into the reagent rack.
4. Manually pipette the Sample, Calibrator, or Controls into the sample well of each reagent cartridge.

Please note that at least 75 µL of sample has to be pipetted manually into the reagent cartridge and 50 µL is needed for each test.

* Before pipetting: Mix the samples using a vortex-type mixer if necessary, and ensure that the samples and sample diluent (if applicable) DO NOT have any bubbles. The same for calibrators and controls.

5. Put the sample rack into the reagent chamber of ACCRE analyzer.
6. Place the assay tips into the reagent chamber of ACCRE analyzer.
7. Input Sample ID, sample type, dilution etc. into the software as directed by the USER MANUAL.
8. Myoglobin (CLIA) assay completed within approximately 15 minutes. Assays will be performed automatically by the ACCRE analyzer.
9. After assay is completed, remove the reagent rack from the ACCRE analyzer. Dispose the used reagent cartridge into an appropriate recipient.

Dilution

Samples with concentrations above 1000 ng/mL can be diluted with negative serum or plasma manually. The recommended dilution is 1:10. And the concentration after dilution should be more than 100ng/mL. After manual dilution, multiply the result by the dilution factor. ACCRE analyzer cannot perform automatic dilution for samples.

Quality control

Each bottle of lyophilized quality control should be re-dissolved by purified water to 1.0mL before use.

Quality control can be performed in accordance with local regulations or requirements related to accreditation, as well as requirements defined in the laboratory's quality control procedure.

RESULTS AND INTERPRETATION

Calculation of Results

The results are automatically calculated by the ACCRE analyzer using stored calibration curve which generate by 3-point calibration, and calculated according to a predefined mathematical model, the results expressed in ng/mL.

Interpretation of Results

The detection range is from 1 ng/mL to 1000 ng/mL. For samples with MYO below the upper limit of detection, quantitative determination can be performed; if the sample is lower than the lower limit of detection, the reported result is <1 ng/mL; if the sample is higher than the upper limit of detection, the reported result is >1000 ng/mL.

Results of Myoglobin (CLIA) assays are determined by testing serum specimens from a study in clinical centers with group of totally 342 healthy adult individuals in China (170 male and 172 female). The 99th percentile value of male is 158.5 ng/mL. The 99th percentile value of female is 108.6 ng/mL.

* Please note that results may differ between laboratories due to variations in

population and test method. If necessary, each laboratory should establish its own reference range.

In interpreting the results, patient's clinical condition including symptoms, diseases history, and other relevant data and information should be considered.

LIMITATIONS

1. If the test results are inconsistent with clinical evidence, additional testing is suggested to confirm the result.
2. For diagnostic purposes, results should be used in conjunction with other data; e.g., symptoms, results of other tests, clinical impressions, etc.
3. Please note that heterophile antibody in human serum or plasma can interfere the test result.
4. High concentration of HAMA can also cause a false positive or false negative result.

PERFORMANCE CHARACTERISTICS

Detection limit

LOB (Limit of Blank) = 0.9 ng/mL

LOB was determined according to CLSI® EP17-A2 recommendations.

Measurement range

The measurement range is the range of values corresponding to the acceptable performance limits (precision and linearity).

The measurement range of the Myoglobin (CLIA) assay is: 1ng/mL to 1000ng/mL.

Linearity

Linearity was evaluated according to CLSI® EP06-A recommendations. The Myoglobin (CLIA) assay is linear between 1 ng/mL to 1000 ng/mL. The absolute linear correlation coefficient(r) should be bigger than 0.9900.

Precision

A precision study was performed according to CLSI® EP05-A3 recommendations.

The Within-lot precision CV% is less than 8%.

The between-lot precision CV% is less than 10%.

Accuracy

The sample which is a control with trueness traceability is used for the detection, and the relative deviation between the detection result and the calibration concentration is within ±10%.

Interfering Substances

Potential interference substances were studied according to CLSI® EP07-A3 recommendations. No significant interference was detected up to the maximum concentrations tested.

Substance	Concentration
Hemoglobin	≤ 500 mg/dL
Bilirubin	≤ 40 mg/dL
Triglyceride	≤ 3000 mg/dL
Total Protein	≤ 5 g/dL
Rheumatoid factor	≤ 1500 IU/mL

HAMA

Patient samples containing human anti-mouse antibodies (HAMA) may give falsely elevated or decreased values. Although HAMA-neutralizing agents are added, extremely high HAMA serum concentrations may occasionally influence results⁵⁻⁹.

Hook effect

No hook effect was found up to Myoglobin (MYO) concentrations of 10000 ng/mL.

WASTE DISPOSAL

Dispose used or unused reagents as well as any other contaminated disposable materials following procedures for infectious or potentially infectious products.

It is the responsibility of each laboratory to handle waste and effluents produced according to their nature and degree of hazardousness and to treat and dispose of them (or have them treated and disposed of) in accordance with any applicable regulations

INDEX OF SYMBOLS

Symbol	Meaning	Symbol	Meaning	Symbol	Meaning
	Consult instructions for use		Contains sufficient for <n> tests		Date of manufacture
	In vitro diagnostic medical device		Use-by date		Do not re-use
	Temperature limit		Batch code		Catalogue number
	Keep away from sunlight		Keep dry		Manufacturer
	Authorized representative in the European Community/ European Union		Do not use if package is damaged and consult instructions for use		Contains biological material of animal origin
	Biological risks		Caution		CE marking
	Upright		Fragile, handle with care		Do not roll
	Control				

LITERATURE REFERENCES

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