i-CHROMA™ Myoglobin

INTENDED USE

i-CHROMA™ Myoglobin along with i-CHROMA™ Reader is a fluorescence immunoassay that measures concentration of myoglobin in human whole blood/serum/plasma.

INTRODUCTION

Myoglobin is an iron- and oxygen-binding protein found in both skeletal and myocardial muscles. It acts as a transport protein and is involved in diffusion of oxygen in the muscle tissue. Myoglobin is a single-chain globular protein of 154 amino acids. It is composed of a central iron-containing ‘Heme’ which is enclosed in a compact bundle-like or prism-like arrangement formed by the eight right-handed α-helices. Being a cytoplasmic protein having low molecular weight (of 17,699 daltons), myoglobin is released into the serum more rapidly as compared to other cardiac markers upon damage to the myocardial cells. Serum concentration of myoglobin increases above the normal range as early as 1 hour after acute myocardial infarction (AMI), attains peak level in approximately 4 to 8 hours after the onset and normalize rapidly afterwards. Thus myoglobin is better suited as a cardiac marker for early diagnosis of AMI. However, the elevated myoglobin is not specific to AMI owing to its large quantities in skeletal muscles as well. Despite its low clinical specificity and weak predictive value towards AMI, myoglobin is still a promising cardiac marker when other markers such as Creatin Kinase Isoenzyme-MB (CK-MB) and Cardiac Troponin-I (cTn-I) are also present as other indicators like clinical signs and ECG are taken into account for diagnosis/confirmation of AMI.8,9

PRINCIPLE

i-CHROMA™ Myoglobin is based on an immunoassay system using antigen-antibody reaction and fluorescence technology. When a test sample and the detection buffer are mixed thoroughly and then loaded in to the sample well on the test cartridge, the complexes of antibody (anti-Myoglobin)-antigen (Myoglobin)-antibody (anti-Myoglobin) produce fluorescence on the membrane of the test cartridge. Thus, more the Myoglobin in test sample, more the complexes that get accumulated on the test cartridge membrane.

i-CHROMA™ Reader scans the intensity of the fluorescence produced on the test cartridge membrane and then displays the Myoglobin concentration on the LCD screen of the reader.

COMPONENTS AND REAGENTS

i-CHROMA™ Myoglobin consists of a Test Cartridge, an ID Chip and a Detection Buffer Tube.

- The test cartridge contains a test strip; on the membrane of which, murine antibodies against human Myoglobin and streptavidin have been immobilized.
- Each test cartridge is individually sealed with a desiccant in an aluminum foil pouch. 25 sealed test cartridges are packed in a box which also contains an ID chip.
- The detection buffer pre-dispensed in a plastic tube contains fluorescent-labeled anti-Myoglobin antibodies, fluorescent biotin-labeled bovine serum albumin (BSA), bovine serum albumin (BSA) as a stabilizer and sodium azide in phosphate buffered saline (PBS) as a preservative.
- 150 µL detection buffer is dispensed in a plastic tube. 25 detection buffer tubes are packed in an aluminum foil pouch which is further packed in a Styrofoam box provided with ice packs for the purpose of shipment.

WARNINGS AND PRECAUTIONS

- For in vitro diagnostic use only.
- Carefully follow the instructions and procedure described in this insert.
- Do not use the test cartridge if its lot number printed on the aluminum foil pouch does not agree with that on the ID chip.
- Do not interchange materials from different product lots; nor use the product beyond the expiration date. Use of a test cartridge beyond the expiration date may offer misleading test results.
- A detection buffer tube should be used for one specimen only. Please discard it after single use.
- The test cartridge should remain sealed in its original pouch until just prior to use. Do not use the test cartridge should the pouch be damaged or the seal broken. Discard it after single use.
- Used test cartridges and detection buffer tubes are potentially infectious. Therefore, the used materials should be handled carefully by appropriate method.
- Do not eat the dehumidifying agent (Silica gel) placed inside the test cartridge pouch.
- Do not smoke, eat or drink in the area where test samples or test reagents are handled.
- i-CHROMA™ Myoglobin is compatible only with i-CHROMA™ Reader.
- i-CHROMA™ Myoglobin as well as the i-CHROMA™ Reader should be used away from vibration and/or magnetic field. During normal usage, i-CHROMA™ Reader may produce minor vibrations, which should be regarded as normal.
- Sodium azide is not likely to be a human health hazard in the quantity present in the i-CHROMA™ Myoglobin detection buffer. Generally, exposure to larger quantities of sodium azide may cause certain health issues like convulsions, low blood pressure and heart rate, loss of consciousness, lung injury and respiratory failure.

STORAGE AND STABILITY

- The detection buffer dispensed in the plastic tube is stable for up to 20 months if stored at 2-8°C.
- i-CHROMA™ Myoglobin test cartridge is stable for up to 20 months (while sealed in the aluminum foil pouch) if stored at 4-30°C.
- If stored in a refrigerator, allow a minimum of 30 minutes for the test cartridges and detection buffer tubes to attain room temperature prior to performing the test.
- Do not remove the test cartridge from the aluminum foil pouch until just prior to use. The test cartridge should be used immediately once removed from the pouch.
- After the test cartridge pouch and the detection buffer tube are opened, i-CHROMA™ Myoglobin should be performed within 30 minutes.

LIMITATIONS OF THE TEST SYSTEM

i-CHROMA™ Myoglobin provides accurate and reliable results subject to the following constraints:

- Use i-CHROMA™ Myoglobin only with i-CHROMA™ Reader.
- Always use freshly collected and/or processed blood samples.
- i-CHROMA™ Myoglobin is meant for single use only. Do not reuse.
- False positive results may occur due to cross-reactions of some components of blood sample with the antibodies and/or non-specific adhesion of some components of blood that have similar epitopes to capture and detect the antibodies.
- In case of false negative results, the most common factor is non-responsiveness of the antigen to the antibodies due to its epitopes being masked by some unknown components such that the antigen cannot be detected by the antibodies.
- The effectiveness of the test is highly dependent on storage of the test kits and test samples at optimal conditions.

Rev.00_121211
MATERIALS SUPPLIED

**REF** CFPC-37

Components of i-CHROMA™ Myoglobin

- **Test Cartridge Box:** 1
  - Sealed Test Cartridges: 25
  - ID Chip: 1
  - Package Insert: 1
- **Aluminum foil pouch containing Detection Buffer Tubes**
  - Pre-filled Detection Buffer Tubes: 25

(*The aluminum foil pouch containing 25 pre-filled detection buffer tubes is delivered separately from the test cartridge box. It is further packed in a Styrofoam box provided with ice packs for the purpose of shipment.)*

MATERIALS REQUIRED BUT SUPPLIED ON DEMAND

Following items can be purchased separately from i-CHROMA™ Myoglobin. Please contact our sales division for more information.

- i-CHROMA™ Reader **REF** FR-203
- i-CHROMA™ Myoglobin Control
- Transfer Pipette (10, 75 µL volume)
- Thermal Printer

**TEST SETUP**

1. Check the contents of i-CHROMA™ Myoglobin: 1ID Chip, 25 Sealed Test Cartridges, and 1 Pouch containing 25 pre-filled Detection Buffer Tubes.
2. Check and ensure that the lot number of the test cartridge matches with that of the ID chip.
3. Keep the i-CHROMA™ Myoglobin test cartridge and detection buffer tube at room temperature for at least 30 minutes just prior to use. Place the test cartridge on a clean, dust-free and flat surface.
4. Turn on the power supply of the i-CHROMA™ Reader.
5. Insert the ID chip into the ID chip port of the i-CHROMA™ Reader.
6. Press the ‘Select’ button on the i-CHROMA Reader. *(Please refer to the ‘i-CHROMA™ Reader Operation Manual’ for complete information and operating instructions.)*

**SAMPLE COLLECTION AND PROCESSING**

The test can be performed on either whole blood or serum or plasma.

- For obtaining the serum sample, collect the blood in a tube without an anticoagulant and allow it to clot. Separate the serum from the clot as soon as possible to avoid hemolysis.
- For obtaining the plasma sample, collect the blood in a tube treated with EDTA, heparin or sodium citrate. Anticoagulants other than EDTA, heparin and sodium citrate have not been evaluated for the purpose of this test.
- If the test cannot be performed within an hour after preparation of the test samples, the serum/plasma should be stored at -20°C.

**TEST PROCEDURE**

1. Transfer 10 µL of whole blood/serum/plasma using a transfer pipette to the detection buffer tube. For performing a quality control test, pipette out 10 µL control standard reagent instead of the sample and transfer it to the detection buffer tube.
2. Mix the sample gently and thoroughly with the detection buffer by pipetting up and down 10 times. Alternatively, mixing may also be effected by vigorous shaking and/or inverting the detection buffer tube many times.
3. Pipette out 75 µL of the above sample mixture and load it into the sample well on the test cartridge.
4. Leave the sample-loaded test cartridge at room temperature for 12 minutes.
5. For scanning the sample-loaded test cartridge, insert it into the test cartridge holder of the i-CHROMA™ Reader. Ensure proper orientation of the test cartridge before pushing it all the way inside the test cartridge holder of the i-CHROMA™ Reader. An arrow has been marked on the test cartridge especially for this purpose.
6. To start scanning, press the ‘Select’ button.
7. i-CHROMA™ Reader will automatically start scanning the sample-loaded test cartridge immediately.
8. Read the test result on the display screen of the i-CHROMA™ Reader.

**INTERPRETATION OF TEST RESULT**

- i-CHROMA™ Reader calculates the test result automatically and displays Myoglobin concentration in ng/mL on the LCD screen.
- The cut-off (reference value) of i-CHROMA™ Myoglobin is 70 ng/mL.
- If the test result is above 70 ng/mL, please consult the physician immediately for detail investigation further.
- The working range of i-CHROMA™ Myoglobin is 5-500 ng/mL.
- i-CHROMA™ Myoglobin test is meant only as a screening tool. In case of a positive result (above 70 ng/mL), consult the physician to discuss the test result. The physician may decide whether to run more tests further.

**QUALITY CONTROL**

- Quality control test should be performed as a part of the good testing practice to confirm the expected quality control results and validity of the assay as well as to ensure accuracy of the test results with clinical samples.
- A quality control test should be performed at regular intervals. Before testing the clinical sample using a new test kit, control standards should be tested to confirm the test procedure, and to verify whether the test produces the expected quality control results. Quality control tests should also be performed whenever there is any question concerning the validity of the results obtained.
- Control standards are not provided with i-CHROMA™ Myoglobin. For more information regarding obtaining the control standards, contact Boditech Med Inc.’s Technical Services for assistance.
- **Internal Control:** i-CHROMA™ Myoglobin has an in-built quality control indicator that satisfies the routine quality control requirements. This internal control test is performed each time a clinical sample is tested. A valid control indicates that the cartridge was inserted and read properly by the i-CHROMA™ Reader. An invalid result from the internal control leads to display an error message on the i-CHROMA™ Reader indicating that the test should be repeated.

**PERFORMANCE CHARACTERISTICS**

1. **Specificity/Interference:** Biomolecules such as heparin, bilirubin, hemoglobin, L-ascorbic acid, glucose were added to the test samples at levels much higher than their normal physiological levels. There was neither any significant interference from these biomolecules with the i-CHROMA™ Myoglobin test measurements nor any significant assay cross-reactivity with these bio-molecules.

2. **Precision:** For testing intra-assay precision, one person tested three different lots of i-CHROMA™ Myoglobin; ten times at each concentration of the control standard. For testing inter-assay precision under the same conditions, three persons tested three different lots of i-CHROMA™ Myoglobin; three times at each concentration of the control standard.

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<th>Whole Blood</th>
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Inter-assay precision of i-CHROMA™ Myoglobin

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3. **Comparability (Correlation):** Myoglobin concentrations of 100 clinical samples were quantified independently with i-CHROMA™ Myoglobin and VIDAS multiparametric immunoassay testing system (BioMerieux Inc., USA). Results of both the test methods were analyzed and their compatibility was investigated with linear regression and coefficient of correlation (R). The coefficient of correlation between the two methods was found to be 0.9891.

Note: Please refer to the table below to identify various symbols

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For technical assistance, please contact:
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E-mail: sales@boditech.co.kr

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**REFERENCES**