

CTNI/Myo/CK-MB FIA Rapid Test Device (Whole Blood/Serum/Plasma) FI2-TMC-001

A rapid test for the diagnosis of myocardial infarction (MI) to detect cardiac Troponin I (cTnI), Myoglobin, and CK-MB in whole blood, serum, or plasma with the use of the Biopanda Fluorescence Immunoassay Analyser. For professional *in vitro* diagnostic use only.

INTENDED USE

The CTNI/Myo/CK-MB FIA Rapid Test Device is intended for *in vitro* quantitative determination of human cardiac Troponin I, myoglobin and CK-MB in whole blood, serum or plasma as an aid in the diagnosis of Myocardial Infarction (MI).

SUMMARY

Cardiac Troponin I is a protein found in cardiac muscle, with a molecular weight of 22.5 kDa.⁷ Troponin I is part of a three subunit complex comprised of Troponin T and Troponin C. Along with tropomyosin, this structural complex forms the main component that regulates the calcium sensitive ATPase activity of actomyosin in striated skeletal and cardiac muscle.⁸ After cardiac injury occurs, Troponin I is released into the blood 4-6 hours after the onset of pain. The release pattern of Troponin I is similar to CK-MB, but while CK-MB levels return to normal after 72 hours, Troponin I remains elevated for 6-10 days, thus providing for a longer window of detection for cardiac injury.

Myoglobin (MYO), Creatine Kinase MB (CK-MB) and cardiac Troponin I (cTnI) are proteins released into the bloodstream after cardiac injury. Myoglobin is a heme-protein normally found in skeletal and cardiac muscle with a molecular weight of 17.8 kDa.¹ When muscle cells are damaged, Myoglobin is released into the blood rapidly due to its relatively small size. The level of Myoglobin increases measurably above baseline within 2-4 hours post-infarct, peaking at 9-12 hours, and returning to baseline within 24-36 hours.^{2,3}

CK-MB is an enzyme also present in the cardiac muscle, with a molecular weight of 87.0 kDa.⁴ Creatine Kinase is a dimeric molecule formed from two subunits designated as "M" and "B", which combine to form three different isoenzymes, CK-MM, CK-BB and CK-MB. CK-MB is the isoenzyme of Creatine Kinase most involved in the metabolism of cardiac muscle tissue.⁵ The release of CK-MB into the blood following an MI can be detected within 3-8 hours after the onset of symptoms. It peaks within 9 to 30 hours, and returns to baseline levels within 48 to 72 hours.⁶

The combined detection of three myocardial injury markers including CK-MB, cTnI and myoglobin has better sensitivity and specificity, which makes the diagnosis more accurate. To achieve early diagnosis, early treatment, reduce missed diagnosis and timely risk stratification of patients.

The CTNI/Myo/CK-MB FIA Rapid Test Device is a simple test that utilizes a combination of antibody coated particles and capture reagent to detect cTnI, Myoglobin, and CK-MB in whole blood, serum or plasma.

PRINCIPLE

The CTNI/Myo/CK-MB FIA Rapid Test Device detects cardiac Troponin I, Myoglobin and CK-MB based on Fluorescence Immunoassay. The sample moves through the strip from sample pad to absorbent pad. If the specimen contains cTnI, Myoglobin or CK-MB, it attaches to the fluorescent microspheres-conjugated specific antibodies. Then the complex will be captured by the capture antibodies coated on the nitrocellulose membrane (Test line). The concentration of cTnI, Myoglobin and CK-MB in the sample correlates with the fluorescence signal intensity captured on the Test line. According to the fluorescence intensity of the test and product standard curve, the concentration of cTnI, Myoglobin and CK-MB in the sample can be calculated by Analyzer to show cTnI, Myoglobin and CK-MB concentration in specimen.

REAGENTS

The test kit includes cTnI antibody coated fluorophores, Myoglobin antibody coated fluorophores, CK-MB antibody conjugated fluorophores and capture reagents coated on the membrane.

PRECAUTIONS

- For professional *in vitro* diagnostic use only.
- Do not use after the expiration date indicated on the package. Do not use the test if the foil pouch is damaged. Do not reuse.
- Avoid cross-contamination of specimens by using a new specimen collection container for each specimen obtained.
- Do not eat, drink or smoke in the area where the specimens and tests are handled. Handle all specimens as if they contain infectious agents. Observe established precautions against microbiological hazards throughout the procedure and follow standard procedures for proper disposal of specimens. Wear protective clothing such as laboratory coats, disposable gloves and eye protection when specimens are assayed.
- Do not interchange or mix reagents from different lots.
- Humidity and temperature can adversely affect results.
- Used testing materials should be discarded in accordance with local regulations.
- Read the entire procedure carefully prior to any testing.
- The Biopanda CTNI/Myo/CK-MB FIA Rapid Test Device should only be used with the Biopanda Fluorescence Immunoassay Device by approved medical professionals.

STORAGE AND STABILITY

- The kit should be stored at 4-30°C until the expiry date printed on the sealed pouch.
- The test must remain in the sealed pouch until use.
- Do not freeze.
- Care should be taken to protect the components of the kit from contamination. Do not use if there is evidence of microbial contamination or precipitation. Biological contamination of dispensing equipment, containers or reagents can lead to false results.

KIT COMPONENTS

- 25 x foil wrapped CTNI/Myo/CK-MB FIA Rapid Test Device
- 25 x Specimen collection tubes with dilution buffer
- 1 x ID card (cTnI)
- Package Insert

MATERIALS REQUIRED BUT NOT PROVIDED

- Timer
- Centrifuge
- Pipette
- Biopanda Fluorescence Immunoassay Device (BR-FIA-2000)

SPECIMEN COLLECTION AND PREPARATION

PREPARATION

- Before performing the test, please make sure that all components are brought to room temperature (15-30°C). Cold buffer solution or moisture condensation on the membrane can lead to invalid test results.
- Take a tube with buffer solution out of the kit. Document patients name or ID on it. Open the screw cap.

BLOOD SAMPLE TAKING

- Collect the specimen according to standard procedures.
- Do not leave specimens at room temperature for prolonged periods. Serum and plasma specimens may be stored at 2-8 °C for up to 1 day, for long term storage, specimens should be kept below -20 °C. Whole blood collected by venipuncture should be stored at 2-8°C if the test is to be run within one day of collection. Do not freeze whole blood specimens. Whole blood collected by finger stick should be tested immediately.
- Bring specimens to room temperature prior to testing. Frozen specimens must be completely thawed and mixed well prior to testing. Avoid repeated freezing and thawing of specimens.
- EDTA K2, Heparin sodium, Citrate sodium and Potassium Oxalate can be used as the anticoagulant for collecting the specimen.

SAMPLE DILUTION / SAMPLE STABILITY

- The specimen (75 µl of whole blood, serum or plasma) can be added directly with the micro pipette into the buffer.
- Close the tube and shake the sample by hand vigorously for approximately 10 seconds to mix the sample and dilution buffer.
- Let the diluted sample rest for approximately 1 minute.
- It is best to place the diluted sample on an ice pack and leave the sample at room temperature for no more than 2 hours.

DIRECTIONS FOR USE

Refer to the Biopanda Fluorescence Immunoassay Device Operation Manual for the complete instructions on use of the Test. The test should be conducted at room temperature.

Allow the test, specimen, buffer and/or controls to reach room temperature (15-30°C) prior to testing.

- Turn on the Analyser. Then according to the user requirement, select "Standard test" or "Quick test" mode.
- Take out the ID card and insert it into the Analyser port.
- Serum/plasma:** Transfer 75 µl of serum/plasma into the buffer tube, mix the specimen and the buffer well.
Whole blood: Transfer 75 µl of whole blood into the buffer tube with pipette; mix the specimen and the buffer thoroughly.
- Add diluted specimen with a Pipette:** Pipette 75 µl of diluted specimen into the sample well of the test device. Start the timer at the same time.
- There are two test modes for the Biopanda Fluorescence Immunoassay Device; Standard Test mode and Quick Test mode. Please refer to the user manual of the Biopanda Fluorescence Immunoassay Device for details.

"Quick test" mode: Insert the test device into the Analyser at 15 minutes after sample application and click "New Test", the Analyser will automatically give the test result after a few seconds.

"Standard test" mode: Insert the test device into the Analyser immediately after sample application, click "New test" at the same time, the Analyser will automatically count down the 15 minutes. After the countdown, the Analyser will give the result at once.

INTERPRETATION OF RESULTS

The result of tests for cTnI, myoglobin and CK-MB are calculated by the Biopanda Fluorescence Immunoassay Device and displays the result on the screen. For additional information, please refer to the user manual of the Biopanda Fluorescence Immunoassay Device.

Linearity range of the Biopanda cTnI FIA Rapid Test is 0.1-40 ng/ml.

Linearity range of the Biopanda Myoglobin FIA Rapid Test is 5-200 ng/mL.
Linearity range of the Biopanda CK-MB FIA Rapid Test Device is 0.2-75 ng/mL.

QUALITY CONTROL

Each Biopanda CTNI/Myo/CK-MB FIA Rapid Test Device contains an internal control that satisfies routine quality control requirements. This internal control is performed each time a patient sample is tested. This control indicates that the test device was inserted and read properly by the Biopanda Fluorescence Immunoassay Analyser. An invalid result from the internal control causes an error message on the Biopanda Fluorescence Immunoassay Analyser indicating that the test should be repeated. An invalid result from the internal control causes an "N/A" message on the Biopanda Fluorescence Immunoassay Analyser. Insufficient specimen volume or incorrect procedural techniques are the most likely reasons for control failure. Review the procedure and repeat the test with a new test. If the problem persists, discontinue using the test kit immediately and contact your local distributor.

LIMITATIONS

- The Biopanda CTNI/Myo/CK-MB FIA Rapid Test Device is for professional *in vitro* diagnostic use, and should only be used for the quantitative detection of cTnI, myoglobin and CK-MB.
- The Biopanda CTNI/Myo/CK-MB FIA Rapid Test Device will only indicate the presence of cTnI, myoglobin and CK-MB in the specimen and should not be used as the sole criterion for evaluating MI.
- Like with all diagnostic tests, a confirmed diagnosis should only be made by a physician after all clinical and laboratory findings have been evaluated.
- High concentrations of Cardiac Troponin I, Myoglobin and CK-MB may produce a dose hook effect, resulting in incorrect interpretation of Cardiac Troponin I, Myoglobin and CK-MB levels. High dose hook effect has not been observed with this test up to 40 ng/mL of Cardiac Troponin I, 200 ng/mL of Myoglobin and 75 ng/mL of CK-MB.
- The hematocrit of the whole blood should be between 25% and 65%.
- The results of the Biopanda CTNI/Myo/CK-MB FIA Rapid Test are based on measuring the levels of cTnI, myoglobin, and CK-MB in a specimen. It should not be used as the sole criterion for treatment decisions. If the result is positive, other clinical findings and alternative test methods are recommended to reach proper medical treatments.

EXPECTED RESULTS

cTnI Conc.	Myo Conc.	CK-MB Conc.	Clinical Reference
<0.5 ng/ml	<90 ng/mL	<5 ng/mL	Not indicative of Acute Myocardial Infarction
<0.5 ng/ml	<90 ng/mL	>5 ng/mL	Indicative of Acute Myocardial Infarction
<0.5 ng/ml	>90 ng/mL	<5 ng/mL	Indicative of Acute Myocardial Infarction
<0.5 ng/ml	>90 ng/mL	>5 ng/mL	Indicative of Acute Myocardial Infarction
>0.5 ng/ml	<90 ng/mL	<5 ng/mL	Indicative of Acute Myocardial Infarction
>0.5 ng/ml	<90 ng/mL	>5 ng/mL	Indicative of Acute Myocardial Infarction
>0.5 ng/ml	>90 ng/mL	<5 ng/mL	Indicative of Acute Myocardial Infarction
>0.5 ng/ml	>90 ng/mL	>5 ng/mL	Indicative of Acute Myocardial Infarction

PERFORMANCE CHARACTERISTICS

1. Accuracy

cTnI: The assay was compared with commercial CLIA test kit with 100 samples. The correlation coefficient(r) is 0.997.

MYO: The assay was compared with commercial CLIA test kit with 100 samples. The correlation coefficient(r) is 0.993.

CK-MB: The assay was compared with commercial Immunosuppression test kit with 100 samples. The correlation coefficient(r) is 0.997.

2. Sensitivity

The Troponin I/Myoglobin/CK-MB (3 in 1) Test Cassette (Whole Blood/Serum/Plasma) can detect levels of Cardiac Troponin I as low as 0.1 ng/mL, Myoglobins as low as 5ng/mL and CK-MB as low as 0.2 ng/mL in whole blood, serum or plasma.

3. Detection range

Troponin I: 0.1~40 ng/mL/Myoglobin: 5~200 ng/mL/CK-MB: 0.2~75 ng/mL

4. Linearity range

Troponin I: 0.1~40 ng/mL, R₂≥0.990/Myoglobin: 5~200 ng/mL, R₂≥0.990/CK-MB: 0.2~75 ng/mL, R₂≥0.990

5. Precision

CV≤15%

6. Cross-reactivity

Cross-reactivity studies were carried out with following analytes.

10,000 ng/mL Skeletal Troponin I, 2,000 ng/mL Troponin T, 20,000 ng/mL Cardiac Myosin, 3,200 ng/mL CK-MM, 1,700 ng/mL CK-BB, HBsAg, HBsAb, HBeAg, HBeAb, HBcAb, syphilis, anti-HIV, anti-H.pylori, MONO, anti-CMV, anti-Rubella and anti-Toxoplasmosis positive specimens.

The results showed no cross-reactivity

7. INTERFERING SUBSTANCES

The following potentially interfering substances were added to cTnI, myoglobin, and CK-MB negative and positive specimens.

Acetaminophen: 20 mg/dl	Caffeine: 20 mg/dl
Acetylsalicylic Acid: 20 mg/dL	Gentisic Acid: 20 mg/dL
Ascorbic Acid: 20mg/mL	Albumin: 10,500mg/dL
Creatin: 200 mg/dL	Hemoglobin 1,000 mg/dL
Bilirubin: 1,000mg/dL	Oxalic Acid: 600mg/dL
Cholesterol: 800mg/dL	Triglycerides: 1,600mg/dL

None of the substances at the concentration tested interfered in the assay.

REFERENCES

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Index of Symbols

	Manufacturer		Tests per kit		Do not reuse test
	<i>In vitro</i> diagnostic medical device		Expiration date		Catalogue number
	Storage temperature		Lot Number		Consult instructions for use
	Do not use if package is damaged				

Thank you for purchasing Biopanda's CTNI/Myo/CK-MB FIA Rapid Test Device. Please read this manual carefully before operating to ensure proper use.



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