



# CTNI/NT-proBNP/D-dimer FIA Rapid Test Device (Whole Blood/Plasma) FI2-TND-001

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

## INTENDED USE

The CTNI/NT-proBNP/D-dimer FIA Rapid Test Device is intended for *in vitro* quantitative determination of human cardiac Troponin I, NT-proBNP, and D-dimer in whole blood or plasma as an aid in the diagnosis of Myocardial Infarction (MI), heart failure, deep vein thrombosis, and pulmonary embolism.

**Note:** Serum specimens should not be used for this test due to low specificity with D-dimer.

## SUMMARY

Cardiac Troponin I is a protein found in cardiac muscle, with a molecular weight of 22.5 kDa.<sup>1</sup> 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.<sup>2</sup> 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.

The N-terminal of the prohormone brain natriuretic peptide (NT-proBNP) is a 76 amino acid N-terminal inactive protein that is cleaved from proBNP to release brain natriuretic peptide. Both BNP and NT-proBNP levels in the blood are used for screening, diagnosis of acute congestive heart failure (CHF) and may be useful to establish prognosis in heart failure, as both markers are typically higher in patients with worse outcome.<sup>5</sup> The plasma concentrations of both BNP and NT-proBNP are also typically increased in patients with asymptomatic or symptomatic left ventricular dysfunction and is associated with coronary artery disease and myocardial ischemia.<sup>6,7</sup>

D-dimer (or D dimer) is a fibrin degradation product (or FDP), a small protein fragment present in the blood after a blood clot is degraded by fibrinolysis. Its formation or increase reflects the activation of coagulation and fibrinolysis system, and its plasma level can represent the production of thrombin active agent fibrin *in vivo*. It can be used as an indicator of thrombosis in the body. The D-dimer content in patients with thrombosis is significantly elevated.<sup>3</sup> In addition, studies have shown that low levels of D-Dimer (100-500 ng/mL) are closely related to the occurrence of cardiovascular diseases, and high levels of D-Dimer may be early exclusion diagnostic indicators for DVT and PE.<sup>4</sup>

The CTNI/NT-proBNP/D-dimer FIA Rapid Test Device is a simple test that utilizes a combination of antibody coated particles and capture reagent to detect cTnI, NT-proBNP, and D-dimer in whole blood or plasma.

## PRINCIPLE

The CTNI/NT-proBNP/D-dimer FIA Rapid Test Device detects cardiac Troponin I, NT-proBNP, and D-dimer based on Fluorescence Immunoassay. The sample moves through the strip from sample pad to absorbent pad. If the specimen contains cTnI, NT-proBNP, or D-dimer, 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, NT-proBNP, and D-dimer 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, NT-proBNP, and D-dimer in the sample can be calculated by Analyzer to show cTnI, NT-proBNP, and D-dimer concentration in specimen.

## REAGENTS

The test kit includes cTnI antibody coated fluorophores, NT-proBNP antibody coated fluorophores, D-dimer 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/NT-proBNP/D-dimer 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/NT-proBNP/D-dimer 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. 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 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.
- Plasma:** Transfer 75 µl of 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, NT-proBNP and D-dimer 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 NT-proBNP is 0.3-22 ng/mL.  
Linearity range of D-Dimer Test is 100-10000 ng/mL

### QUALITY CONTROL

Each Biopanda CTNI/NT-proBNP/D-dimer 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 Device. An invalid result from the internal control causes an error message on the Biopanda Fluorescence Immunoassay Device indicating that the test should be repeated. An invalid result from the internal control causes an "N/A" message on the Biopanda Fluorescence Immunoassay Device. 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/NT-proBNP/D-dimer FIA Rapid Test Device is for professional *in vitro* diagnostic use, and should only be used for the quantitative detection of cTnI, NT-proBNP and D-dimer.
- The Biopanda CTNI/NT-proBNP/D-dimer FIA Rapid Test Device will only indicate the presence of cTnI, NT-proBNP and D-dimer in the specimen and should not be used as the sole criterion for evaluating MI, heart failure, DVT, or PE.
- 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, NT-proBNP and D-dimer 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, 40 ng/mL of Cardiac Troponin I, 22 ng/mL of NT-proBNP, and 10,000 ng/mL of D-Dimer.
- The hematocrit of the whole blood should be between 25% and 65%.
- The results of the Biopanda CTNI/NT-proBNP/D-dimer FIA Rapid Test are based on measuring the levels of cTnI, NT-proBNP and D-dimer 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.	NT-proBNP Conc.	D-dimer Conc.	Clinical Reference
<0.5 ng/ml	<0.45 ng/mL	<500 ng/mL	Not indicative of Acute Myocardial Infarction, Heart Failure, DVT and PE
<0.5 ng/ml	>0.45 ng/mL	<500 ng/mL	Indicative of Acute Heart Failure
<0.5 ng/ml	<0.45 ng/mL	>500 ng/mL	Indicative of DVT and PE
<0.5 ng/ml	>0.45 ng/mL	>500 ng/mL	Indicative of Acute Heart Failure, DVT and PE
>0.5 ng/ml	<0.45 ng/mL	<500 ng/mL	Indicative of Acute Myocardial Infarction
>0.5 ng/ml	>0.45 ng/mL	<500 ng/mL	Indicative of Acute Myocardial Infarction, Heart Failure
>0.5 ng/ml	<0.45 ng/mL	>500 ng/mL	Indicative of Acute Myocardial Infarction, DVT and PE
>0.5 ng/ml	>0.45 ng/mL	>500 ng/mL	Indicative of Acute Myocardial Infarction, Heart Failure, DVT and PE

### PERFORMANCE CHARACTERISTICS

#### 1. METHOD COMPARISON

The assay was compared with commercial test kit for each test item independently. For cTnI test item, 105 specimens were tested and the correlation coefficient (R2) is 0.9704. For D-Dimer test item 115 specimens were tested and the correlation coefficient (R2) is 0.9757. For NT-proBNP test item 98 specimens were tested and the correlation coefficient (R2) is 0.9825.

#### 2. ACCURACY

The test deviation is  $\leq \pm 15\%$ .

#### 3. SENSITIVITY

The CTNI/NT-proBNP/D-dimer FIA Rapid Test Device can detect levels of Cardiac Troponin I as low as 0.1ng/mL, NT-proBNP as low as 0.3 ng/mL, and D-Dimer as low as 100 ng/mL and in whole blood or plasma.

#### 4. DETECTION RANGE

Troponin I: 0.1-40 ng/mL.  
NT-proBNP: 0.3-22 ng/mL.  
D-Dimer: 100-10,000 ng/mL.

#### 5. LINEARITY RANGE

Troponin I: 0.1-40 ng/mL,  $R \geq 0.990$ .  
NT-proBNP: 0.3-22 ng/mL,  $R \geq 0.990$ .  
D-Dimer: 100-10,000 ng/mL,  $R \geq 0.990$ .

#### 6. PRECISION

C.V.  $\leq 15\%$

#### 7. CROSS-REACTIVITY

Cross-reactivity studies were carried out with following analytes. 10,000 ng/mL Skeletal Troponin I, 2,000 ng/mL Troponin T, HBsAg, 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.

#### 8. INTERFERING SUBSTANCES

The following potentially interfering substances were added to cTnI, NT-proBNP and D-dimer negative and positive specimens.

Acetaminophen: 20 mg/dl  
Acetylsalicylic Acid: 20 mg/dL  
Ascorbic Acid: 20mg/mL  
Creatin: 200 mg/dL  
Bilirubin: 1,000mg/dL  
Cholesterol: 800mg/dL  
None of the substances at the concentration tested interfered in the assay.

Caffeine: 20 mg/dL  
Gentisic Acid: 20 mg/dL  
Albumin: 10,500mg/dL  
Hemoglobin 1,000 mg/dL  
Oxalic Acid: 600mg/dL  
Triglycerides: 1,600mg/dL

### REFERENCES

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- Nakamura T, Sakamoto K, Yamano T, Kikkawa M, Zen K, Hikosaka T, Kubota T, Azuma A, Nishimura T (May 2002). "Increased plasma brain natriuretic peptide level as a guide for silent myocardial ischemia in patients with non-obstructive hypertrophic cardiomyopathy". *J. Am. Coll. Cardiol.* 39 (10): 1657-63.

### 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/NT-proBNP/D-dimer FIA Rapid Test Device. Please read this manual carefully before operating to ensure proper use.



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