High Sensitivity NT-proBNP FIA Rapid Test Device

(Whole Blood/Serum/Plasma)

FI2-PBNP-002

A rapid test for the diagnosis of heart failure to detect NT-proBNP quantitatively in whole blood, serum or plasma with the use of the Biopanda Fluorescence Immunoassay Device (BR-FIA-2000).
For professional in vitro diagnostic use only.

INTENDED USE

The High Sensitivity NT-proBNP FIA Rapid Test Device is based on Fluorescence Immunoassay for the quantitative determination of human NT-proBNP in whole blood, serum or plasma as an aid in the diagnosis of heart failure.

SUMMARY

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.[1] 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.[2.3.]

The High-Sensitivity NT-proBNP FIA Rapid Test Device utilizes a combination of anti-NT-proBNP antibody coated particles and capture reagents to quantitatively detect NT-proBNP in whole blood, serum or plasma. The minimum detection level is 30 pg/mL.

PRINCIPLE

The High Sensitivity NT-proBNP FIA Rapid Test Device detects NT-proBNP based on Fluorescence Immunoassay. The sample moves through the strip from sample pad to absorbent pad. If the specimen contains NT-proBNP, it attaches to the fluorescent microspheres-conjugated anti-NT-proBNP antibodies. Then the complex will be captured by the capture antibodies coated on the nitrocellulose membrane (Test line). The concentration of NT-proBNP in the sample correlates linearly with the fluorescence signal intensity captured on the T line. According to the fluorescence intensity of the test and standard curve, the concentration of NT-proBNP in the sample can be calculated by the Biopanda Fluorescence Immunoassay Device (BR-FIA-2000) to show NT-proBNP concentration in specimen.

REAGENTS

The test includes anti-NT-proBNP antibody conjugated fluorophores and capture reagents coated on the membrane.

PRECAUTIONS

1. For professional *in vitro* diagnostic use only.
2. 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.
3. Avoid cross-contamination of specimens by using a new specimen collection container for each specimen obtained.
4. 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.
5. Do not interchange or mix reagents from different lots.
6. Humidity and temperature can adversely affect results.
7. Used testing materials should be discarded in accordance with local regulations.
8. Read the entire procedure carefully prior to any testing.
9. The Biopanda High Sensitivity NT-proBNP FIA Rapid Test Device should only be used with the Biopanda Fluorescence Immunoassay Device by approved medical professionals.

STORAGE AND STABILITY

1. The kit should be stored at 4-30°C until the expiry date printed on the sealed pouch.
2. The test must remain in the sealed pouch until use.
3. Do not freeze.
4. 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 NT-proBNP test devices
- 25 x Specimen collection tubes with extraction buffer
- 1 x ID card (High Sensitivity NT-proBNP)
- Package Insert

MATERIALS REQUIRED BUT NOT PROVIDED

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

SPECIMEN COLLECTION AND PREPARATION

For Venipuncture Specimens

1. Collect the specimen according to standard procedures.
2. Separate the serum or plasma from blood as soon as possible to avoid hemolysis. Only clear, non-hemolyzed specimens can be used.
3. Do not leave specimens at room temperature for prolonged periods. Serum and plasma specimens may be stored at 2-8 °C for up to 3 days, 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 2 days of collection. Do not freeze whole blood specimens. Whole blood collected by fingerstick should be tested immediately.
4. 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.
5. EDTA K2, Heparin sodium can be used as the anticoagulant for collecting the specimen. A clean tube without anticoagulants can be used to collect serum specimens.

For Finger-pricked Whole Blood Specimens, please refer to the DIRECTIONS FOR USE for further information.

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. Cold buffer solution or moisture condensation on the membrane can lead to invalid test results.

Note: There are two test modes for the Biopanda Fluorescence Immunoassay Device: Standard Test mode and Quick Test mode. *Standard Test* mode is a 'set and forget' method where the test will automatically be read after 15 minutes. *Quick* Test mode provides an instant result but the user must monitor the 15 minute test time themselves. It is suitable when running multiple tests concurrently.
Refer to the Biopanda Fluorescence Immunoassay Device Operation Manual for further details.

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

1. Turn on the Analyser. Then according to users' requirements, select "Standard test" or "Quick test" mode.
2. Take out the ID card and insert it into the Analyser port.
3. Remove the test cassette from the sealed foil pouch and start testing as soon as possible.
4. Follow the appropriate steps below for the chosen specimen type:
   - **a.** **<u>For venipuncture whole blood/serum/plasma specimens:</u>**
     1. Pipette **40 µl of whole blood/serum/plasma** into the buffer tube.
     2. Close the tube cap and shake the tube for approximately **10 seconds** to mix the specimen and dilution buffer well.
     3. Let the diluted specimen homogenise for approximately 1 minute. Diluted specimens should be used as soon as possible.
     4. **Pipette 75 µl of diluted specimen** into the specimen well (S) of the test cassette. Start the timer at the same time.
   - **b.** **<u>For finger-prick whole blood specimens:</u>**
     i. Wash hands with soap and warm water or clean finger with an alcohol pad. Allow to dry.
     ii. Massage the hand without touching the puncture site by applying pressure down the hand towards the finger to be pricked. The middle or ring finger is recommended.
     iii. Use a sterile lancet to puncture the skin. Wipe away the first sign of

blood.

iv. Gently apply pressure from palm to the pricked finger so a rounded drop of blood forms over the puncture site.

v. Using the provided capillary dropper and ensuring the dropper is level, touch the open end to the rounded drop of blood without squeezing the dropper bulb. The dropper will automatically collect the correct volume of blood (approx. 40 µl), *see Figure 1 below*.

vi. Dispense the whole blood specimen into the buffer tube by squeezing the dropper bulb.

vii. Close the tube cap and shake the tube for approximately **10 seconds** to mix the specimen and dilution buffer well.

viii. Using a disposable dropper, **draw the diluted specimen to the fill line** marked on the dropper (approx. 75 µl), then add to the specimen well (S) of the test cassette. Start the timer.

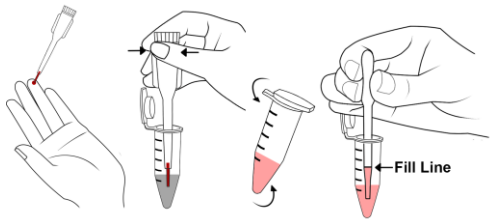

Figure 1

5. Test results should be read at **15 minutes** with the use of the Biopanda Fluorescence Immunoassay Analyser.

INTERPRETATION OF RESULTS

The result of tests for High Sensitivity NT-proBNP is 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 (BR-FIA-2000).
Linearity range of the Biopanda High Sensitivity NT-proBNP FIA Rapid Test is 30-30000 pg/mL.

QUALITY CONTROL

Each Biopanda High Sensitivity NT-proBNP 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

1. The Biopanda High Sensitivity NT-proBNP FIA Rapid Test Device is for professional *in vitro* diagnostic use, and should only be used for the quantitative detection of NT-proBNP.
2. The Biopanda High Sensitivity NT-proBNP FIA Rapid Test Device will only indicate the presence of NT-proBNP in the specimen and should not be used as the sole criterion for evaluating AMI.
3. As with all diagnostic tests, a confirmed diagnosis should only be made by a physician after all clinical and laboratory findings have been evaluated.
4. High concentrations of NT-proBNP may produce a dose hook effect, resulting in incorrect interpretation of NT-proBNP levels. High dose hook effect has not been observed with this test up to 30000 pg/mL of NT-proBNP.
5. The haematocrit level of the whole blood should be between 25% and 65%.
6. The results of the Biopanda High Sensitivity NT-proBNP FIA Rapid Tests are based on measuring the levels of NT-proBNP 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

Concentrations	Clinical Reference
<450 pg/mL	Not indicative of Acute Heart Failure
>450 pg/mL	Indicative of Acute Heart Failure

PERFORMANCE CHARACTERISTICS

1. **ACCURACY**: The test deviation is ≤±15%.
2. **SENSITIVITY**: The Biopanda High Sensitivity NT-proBNP FIA Rapid Test Device can detect levels of NT-proBNP as low as 30 pg/mL in whole blood, serum or plasma.
3. **DETECTION RANGE**: 30-30000 pg/mL
4. **LINEARITY RANGE**: 30-30000 pg/mL, R≥0.990
5. **PRECISION**

INTRA-LOT PRECISION

Within-run precision has been determined by using 10 replicates of 2 specimens of NT-proBNP. C.V. is ≤ 15%.

INTER-LOT PRECISION

Between-run precision has been determined by using 10 replicates for each of three lots using 2 specimens of NT-proBNP. C.V. is ≤ 15%.

6. **CROSS-REACTIVITY**

Cross-reactivity studies were carried out with following analytes.
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 NT-proBNP negative and positive specimens, respectively.

Acetaminophen: 20 mg/dL	Caffeine:20 mg/dL
Acetylsalicylic Acid:20 mg/dL	Gentisic Acid:20 mg/dL
Ascorbic Acid:20mg/dL	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

8. **METHOD COMPARISON**

The product was evaluated with 126 clinical samples compared with commercial CLIA test kit. The correlation coefficient(r) is 0.9882.

REFERENCES

1. Bhalla V, Willis S, Maisel AS (2004). "B-type natriuretic peptide: the level and the drug--partners in the diagnosis of congestive heart failure". Congest Heart Fail 10 (1 Suppl 1): 3–27.
2. Atisha D, Bhalla MA, Morrison LK, Felicio L, Clopton P, Gardetto N, Kazanegra R, Chiu A, Maisel AS (September 2004). "A prospective study in search of an optimal B-natriuretic peptide level to screen patients for cardiac dysfunction". Am. Heart J. 148 (3): 518–23.
3. 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	Σ	Tests per kit	2	Do not reuse test
IVD	*In vitro* diagnostic medical device		Expiration date	REF	Catalogue number
4°C 30°C	Store between 4-30°C	LOT	Lot Number		Consult instructions for use
	Do not use if package is damaged				

Thank you for purchasing Biopanda's High Sensitivity NT-proBNP FIA Rapid Test Device. Please read this manual carefully before operating to ensure proper use.

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