Document No. : INS-NB_G-EN (Rev. 04)
Revision date : April 01, 2022

Cardiac

AFIAS NT-proBNP

INTENDED USE

AFIAS NT-proBNP is a fluorescence immunoassay (FIA) for the quantitative determination of NT-proBNP in human whole blood/serum/plasma. It is useful as an aid in the diagnosis of persons suspected of having congestive heart failure.

For *in vitro* diagnostic use only.

INTRODUCTION

N-terminal pro-brain natriuretic peptide (NT-proBNP) is produced predominately by the cardiac ventricular myocytes [1] and is released in response to myocardial stress and filling pressure[2] and is involved in maintaining intravascular volume homeostasis[3,4]. After stimulation of heart muscle cells, the natriuretic peptides are produced as prohormones (proBNP) and this is cleaved into two fragments which are secreted into the bloodstream as the 32 amino acids active BNP and the N-terminal fragment of 76 amino acids designated as NT-proBNP. NT-proBNP immunoassays are widely used and are now considered to be a useful marker and have a high degree of diagnostic accuracy in clinical practice and cardiovascular research as a diagnostic tool for the occurrence and severity of heart failure (HF) [5,6,7]. Therefore NT-proBNP measurements in human blood are helpful not only for the cardiac disease diagnosis but also for evaluation of patients with suspected HF and assessment of severity of the disease.

PRINCIPLE

The test uses a sandwich immunodetection method. The detector antibodies and biotinylated antibodies in buffer bind to antigens in the sample, forming biotinylated antibody-antigen-detector antibody complexes, and migrate onto nitrocellulose matrix to be captured by the other immobilized-streptavidin on a test strip.

The more antigen in sample will form the more biotinylated antibody-antigen-detector antibody complex which lead to stronger fluorescence signal by detector antibodies, which is processed by the instrument for AFIAS tests to show NT-proBNP concentration in the sample.

COMPONENTS

AFIAS NT-proBNP consists of 'cartridges'.

- Each sealed aluminum pouch contains two cartridges.
- The cartridge part contains the membrane called a test strip which has streptavidin at the test line and chicken IgY at the control line.
- The detector part has 2 granules containing the anti-NT-proBNP fluorescence conjugate, anti-NT-proBNP biotin conjugate and anti-chicken IgY-fluorescence conjugate, HAMA blocker, bromophenol blue, bovine serum albumin (BSA) and sucrose as a stabilizer and sodium azide as a preservative in Tris-Cl buffer.
- The diluent part contains tween 20 as surfactant and NaCl in MES buffer.

WARNINGS AND PRECAUTIONS

- For *in vitro* diagnostic use only.
- Follow instructions and procedures described in this 'Instructions for use'.
- Use only fresh samples and avoid direct sunlight.
- Lot numbers of all the test components (cartridge and ID chip) must match each other.
- Do not interchange the test components between different lots or use the test components after the expiration date, either of which might yield incorrect test result(s).
- Do not reuse cartridges. A cartridge should be used for testing one sample only.
- The cartridge should remain sealed in its original pouch until just before use. Do not use a cartridge, if the pouch is damaged or has already been opened.
- Frozen sample should be thawed only once. For shipping, samples must be packed in accordance with local regulations. Sample with severe hemolysis and/or hyperlipidemia must not be used.
- If test components and/or sample are stored in refrigerator, then allow cartridge and sample to be at room temperature for approximately 30 minutes before use.
- The instrument for AFIAS tests may generate slight vibration during use.
- Used cartridges, and pipette tips should be handled carefully and discarded by an appropriate method in accordance with relevant local regulations.
- The cartridge contains sodium azide (NaN_3), and they may cause certain health issues like convulsions, low blood pressure and heart rate, loss of consciousness, lung injury and respiratory failure. Avoid contact with skin, eyes, and clothing. In case of contact, rinse immediately with running water.
- No Biotin interference was observed in **AFIAS NT-proBNP** when biotin concentration in the sample was below 10 ng/mL. If a patient has been taking biotin at dosage of more than 0.03 mg a day, it is recommended to test again 24 hours after discontinuation of biotin intake.
- **AFIAS NT-proBNP** will provide accurate and reliable results subject to the below conditions.
  - **AFIAS NT-proBNP** should be used only in conjunction with the instrument for AFIAS tests.
  - Have to use recommended anticoagulant.

Recommended anticoagulant
K_2 EDTA, K_3 EDTA, Sodium heparin, Lithium heparin

LIMITATIONS OF THE TEST SYSTEM

- The test may yield false positive result(s) due to the cross-reactions and/or non-specific adhesion of certain sample components to the capture/detector antibodies.
- The test may yield false negative result(s) due to the non-responsiveness of the antigen to the antibodies which is the most common if the epitope is masked by some unknown components, so therefore not being able to be detected or captured by the antibodies. The instability or degradation of the antigen with time and/or temperature may also cause false negative result as it makes the antigen unrecognizable by the antibodies.
- Other factors may interfere with the test and cause erroneous results, such as technical/procedural errors, degradation of the test components/reagents or presence of interfering substances in the test samples.
- Any clinical diagnosis based on the test result must be supported by a comprehensive judgment of the concerned physician in conjunction with clinical symptoms and other relevant test results.

STORAGE AND STABILITY

Storage condition			
Component	Storage Temperature	Shelf life	Note
Cartridge	2 - 30 °C	20 months	Unopened
		1 month	Resealed

- Return an unused cartridge to the spare cartridge zipper bag containing the desiccant pack. Reseal along entire edge of zip-seal.

MATERIALS SUPPLIED

REF SMFP-36

Components of **AFIAS NT-proBNP**

- Cartridge box
  - Cartridge 24
  - Pipette Tip (Zipper bag) 24
  - Spare Cartridge zipper bag 1
  - ID Chip 1
  - Instruction For Use 1

MATERIALS REQUIRED BUT SUPPLIED ON DEMAND

Following items can be purchased separately from **AFIAS NT-proBNP**.

Please contact our sales division for more information.

- **Instrument for AFIAS tests**
  - **AFIAS-1** REF FPRR019
  - **AFIAS-3** REF FPRR040
  - **AFIAS-6** REF FPRR020
  - **AFIAS-10** REF FPRR038
- **Boditech Cardiac Control** REF CFPO-98
- **Boditech Cardiac Calibrator** REF CFPO-110
- **Boditech NT-proBNP Control** REF CFPO-245
- **Boditech NT-proBNP Calibrator** REF CFPO-271

SAMPLE COLLECTION AND PROCESSING

The sample type for **AFIAS NT-proBNP** is human whole blood/serum/plasma.

- It is recommended to test the sample within 24 hours after collection.
- The samples (serum, plasma) should be separated from the clot by centrifugation within 3 hours after the collection of whole blood.
- The samples (whole blood, serum, plasma) may be stored for a week at 2-8 °C prior to being tested. If testing will be delayed more than a week, samples should be frozen at -20 °C.
- The samples (serum, plasma) stored frozen at -20 °C for 3 months showed no performance difference.
- However, the whole blood sample should not be kept in a freezer in any case.
- As a repeated freeze-thaw cycle may affect the test result, do not refreeze previously frozen samples.

TEST SETUP

- Check the components of the **AFIAS NT-proBNP** as described below.: Cartridges, pipette tips, an ID chip, a spare cartridge zipper bag and an instructions for use.
- If the sealed cartridge has been stored in a refrigerator, place them on a clean and flat surface at room temperature for at least 30 minutes before testing.
- Turn on the instrument for AFIAS tests.
- Empty the tip box.
- Insert the ID chip into the 'ID chip port'.

※ **Please refer to the instrument for AFIAS tests operation manual for complete information and operating instructions.**

TEST PROCEDURE

▶ **AFIAS-1, AFIAS-3, AFIAS-6**

General mode

1) Insert a cartridge into the cartridge holder.
2) Insert a tip into the tip hole of the cartridge.
3) Select the 'General mode' in the instrument for AFIAS tests.
4) Take 100 µL of the sample (whole blood/serum/plasma/control) using a pipette and dispense it into the sample well of the cartridge.
5) Tap the 'Start' button on the screen.
6) The test result will be displayed on the screen after 12 minutes.

▶ **AFIAS-10**

Normal mode

1) Insert a cartridge into the cartridge holder.
2) Insert a tip into the tip hole of the cartridge.
3) Tap the 'Load' button of the bay that holds the cartridge with the tip to read the barcode of the cartridge and please confirm the item name written on the cartridge.
4) Insert the sample tube into the tube rack.
5) Insert the tube rack into the loading part of the sampling station.
6) Tap the 'Start' button on the screen.
7) The test result will be displayed on the screen after 12 minutes.

Emergency mode – General tip

1) The test procedure is same with the 'Normal mode 1) – 3)'.
2) Convert the 'Emergency mode' in AFIAS-10.
3) Select the tip type (general tip) on the screen.
4) Select the sample type (whole blood/serum/plasma) on the screen.
5) Take 100 µL of the sample using a pipette and dispense it into the sample well of the cartridge.
6) Tap the 'Start' button on the screen.
7) The test result will be displayed on the screen after 12 minutes.

INTERPRETATION OF TEST RESULT

- The instrument for AFIAS tests calculates the test result automatically and displays NT-proBNP concentration of the test sample in terms of pg/mL.
- Reference value: 125 pg/mL
- Working range: 10-30,000 pg/mL.

QUALITY CONTROL

- Quality control tests are a part of the good testing practice to confirm the expected results and validity of the assay and should be performed at regular intervals.
- Quality control tests should also be performed whenever there is any question concerning the validity of the test results.
- Control materials are not provided on demand with **AFIAS NT-proBNP**. For more information regarding obtaining the control materials, contact Boditech Med Inc.'s Sales Division for assistance.
  (Please refer to the instructions for use of control material.)

PERFORMANCE CHARACTERISTICS

- **Analytical sensitivity**

Limit of Blank (LoB)	5.94 pg/mL
Limit of Detection (LoD)	8.03 pg/mL
Limit of Quantification (LoQ)	10.0 pg/mL

- **Analytical specificity**

- Cross-reactivity
  Biomolecules such as below the ones in the table were added to the test sample(s) at concentrations much higher than their normal physiological levels in the blood. **AFIAS NT-proBNP** test results did not show any significant cross-reactivity with these biomolecules.

Cross reactivity materials	Concentration
Troponin Complex	1.0 ug/mL
CK-MB	1.0 ug/mL
Myoglobin	3.5 ug/mL
BNP	3.5 ug/mL
CNP	3.5 ug/mL
NT-proANP	3.5 ug/mL
Endothelin	20 pg/mL
D-dimer	100 ug/mL
Adrenomedullin	1.0 ng/mL
Aldosterone	0.6 ng/mL
Angiotensin I	0.6 ng/mL
Angiotensin II	0.6 ng/mL

- Interference
  Interferents listed in the following table were added to the test sample at the concentration mentioned below. **AFIAS NT-proBNP** test results did not show any significant interference with these materials.

Interference materials	Concentration
D-glucose	60 mM/L
L-Ascorbic acid	0.2 mM/L
Bilirubin(conjugated)	0.4 mM/L
Hemoglobin	2 g/L
Cholesterol	13 mM/L
Triglyceride	10 mg/ml
EDTA_K2	10.8 mg/ml
EDTA_K3	10.8 mg/ml
Sodium Heparin	54 mg/ml
Lithium Heparin	54 mg/ml

- **Precision**

- Single-site study
  Repeatability (within-run precision)
  within-laboratory precision (Total precision)
  Lot to lot precision
  3 Lots of **AFIAS NT-proBNP** were tested for 20 days. Each standard material was tested 2 times per day. For each test, each material was duplicated.

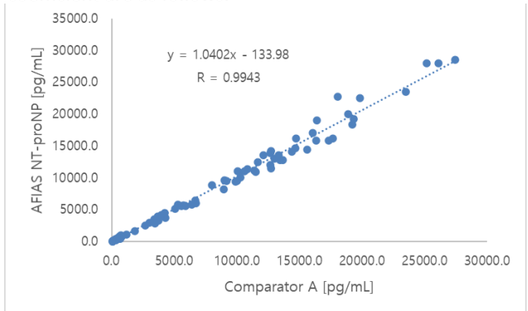
- Multi-site study
  Reproducibility
  1 Lot of **AFIAS NT-proBNP** was tested for 5 days in 3 different sites (1 person per 1 site, 1 instrument per 1 site). Each standard material was tested 1 time per and 5 replicates per day.

NT-proBNP [pg/mL]	Repeatability		Total precision	
	AVG	CV (%)	AVG	CV (%)
63.35	63.91	5.34	63.40	5.54
292.55	296.62	5.90	295.38	6.33
2259.7	2273.50	5.89	2267.92	5.75
NT-proBNP [pg/mL]	Lot to lot precision		Reproducibility	
	AVG	CV (%)	AVG	CV (%)
63.35	63.92	5.73	64.08	6.14
292.55	291.18	5.48	290.71	5.67
2259.7	2258.95	6.26	2265.97	5.55

- **Accuracy**
  The accuracy was confirmed by testing with 3 different lots of **AFIAS NT-proBNP**. The tests were repeated 10 times at each concentration of the control standard.

NT-proBNP [pg/mL]	Lot 1	Lot 2	Lot 3	Mean	Recovery (%)
63.35	62.61	63.94	64.14	63.56	100.3
502.62	482.78	512.83	508.46	501.36	99.7
941.89	940.55	945.72	947.61	944.63	100.3
1381.16	1383.11	1417.66	1367.13	1389.30	100.6
1820.43	1809.36	1804.94	1868.20	1827.50	100.4
2259.70	2198.67	2235.25	2322.61	2252.18	99.7

- **Comparability**
  NT-proBNP concentration of 104 clinical samples were quantified independently with **AFIAS NT-proBNP (AFIAS-6)** and **comparator A** as per prescribed test procedures. Test results were compared, and their comparability was investigated with linear regression and correlation coefficient (R). The regression equation and correlation coefficient are as follows.



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

Σ	Sufficient for <n> tests
	Read instruction for use
	Use by Date
LOT	Batch code
REF	Catalog number
	Caution
	Manufacturer
EC REP	Authorized representative of the European Community
IVD	In vitro diagnostic medical device
	Temperature limit
	Do not reuse
CE	This product fulfills the requirements of the Directive 98/79/EC on in vitro diagnostic medical devices

For technical assistance, please contact:
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