Cardiac

ichroma™ NT-proBNP

INTENDED USE

ichroma™ 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 predominantly 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; dried detector antibody in the buffer binds to antigen in the sample, forming antigen-antibody complexes, and migrates onto the nitrocellulose matrix to be captured by the other immobilized-antibodies on test strip.

The more antigen in sample forms the more antigen-antibody complex and leads to stronger intensity of fluorescence signal on detector antibody, which is processed by instrument for ichroma™ tests to show NT-proBNP concentration in sample.

COMPONENTS

ichroma™ NT-proBNP consists of 'Cartridges', 'Detectors', 'Diluent', 'Capillary tubes' and an 'ID chip'.

- The cartridge contains a test strip, the membrane which has anti human NT-proBNP at the test line, while chicken IgY at the control line.
- Each cartridge is individually sealed in an aluminum foil pouch containing a desiccant. 25 sealed cartridges are packed in a box which also contains an ID chip and 25 sealed capillary tubes.
- The detector contains anti human NT-proBNP-fluorescence conjugate, anti chicken IgY-fluorescence conjugate, bovine serum albumin (BSA) as a stabilizer and sodium azide as a preservative in phosphate buffered saline.
- Each detector contains a granule. 25 tubes of detector are packed in a pouch and packed in a box with 5 ml of diluent.

WARNINGS AND PRECAUTIONS

- For *in vitro* diagnostic use only.
- Follow the instructions and procedures described in this 'Instruction for use'.
- Use only fresh samples and avoid direct sunlight.
- Lot numbers of all the test components (cartridge, ID chip, detector and diluent) 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 or detection buffer tubes. A detection buffer tube should be used for processing of one sample only. 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 the cartridge, if 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.
- Allow cartridge, detection buffer and sample to be at room temperature for approximately 30 minutes before use.
- The instrument for ichroma™ tests may generate slight vibration during use.
- Used detectors, pipette tips and cartridges should be handled carefully and discarded by an appropriate method in accordance with relevant local regulations.
- An 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.
- **ichroma™ NT-proBNP** will provide accurate and reliable results subject to the the below conditions.
  - **ichroma™ NT-proBNP** should be used only in conjunction with instrument for ichroma™ tests.
  - Have to use recommended anticoagulant sample.

Sample type	Recommended anticoagulant
Whole blood	K_2 EDTA, K_3 EDTA
Plasma	Sodium heparin, Lithium heparin
Serum	Not applicable

STORAGE AND STABILITY

- The cartridge is stable for 20 months (while sealed in an aluminum foil pouch) if stored at 4-30 °C.
- The detector and the diluent is stable for 20 months if stored at 2-8 °C.
- After the cartridge pouch is opened, the test should be performed immediately.

LIMITATION 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 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 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 including clinical symptoms and other relevant test results.

MATERIALS SUPPLIED

REF CFPC-77

Components of **ichroma™ NT-proBNP**

- Cartridge Box:
  - Cartridges 25
  - 50 µL Capillary tube 25
  - ID Chip 1

- Instruction For Use 1

▪ Buffer Box

✓ For ichroma™ II

- Detectors (Capped with plastic lid) 25
- Diluent 1

✓ For ichroma™-50

- Detectors (Sealed with aluminum foil) 25
- Diluent 1

MATERIALS REQUIRED BUT SUPPLIED ON DEMAND

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

Please contact our sales division for more information.

- Instrument for ichroma™ tests
  - **ichroma™ II** REF FPRR021
  - **ichroma™-50** REF FPRR027
- **i-Chamber** REF FPRR009

SAMPLE COLLECTION AND PROCESSING

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

- It is recommended to test the sample within 24 hours after collection.
- The serum or plasma should be separated from the clot by centrifugation within 3 hours after the collection of whole blood. If longer storage is required, e.g. if the test could not be performed within 24 hours, serum or plasma should be immediately frozen below -20 °C. The freezing storage of sample up to 3 months does not affect the quality of results.
- However, the whole blood sample should not be kept in a freezer in any case.
- Once the sample was frozen, it should be used one time only for test, because repeated freezing and thawing can result in the change of test values.

TEST SETUP

- Check the contents of **ichroma™ NT-proBNP**: Sealed Cartridge, Detectors, Diluent, Capillary tubes and ID Chip.
- Ensure that the lot number of the cartridge matches that of the ID chip as well as the buffer box.
- Keep the sealed cartridge (if stored in refrigerator) and the buffer box at room temperature for at least 30 minutes just prior to the test. Place the cartridge on a clean, dust-free and flat surface.
- Turn on the i-Chamber and set temperature to 25°C. To reach 25°C , it takes approximately 5-10 minutes. The necessary time may vary depending on environmental conditions.
- Turn on the instrument for ichroma™ tests.
  (Please refer to the 'Instrument for ichroma™ tests Operation Manual' for complete information and operating instructions.)

TEST PROCEDURE

▶ **ichroma™ II**

1) Transfer 150 µL of diluent using a pipette to a tube containing detector.
2) Transfer 50 µL of sample (Human whole blood/ serum/ plasma/control) to the detector tube.
3) Close the lid of the detector tube and mix the sample thoroughly by shaking about 20 times.
4) Pipette out 75 µL of a sample mixture and load it into the sample well on the cartridge.
5) Insert the sample-loaded cartridge into the i-Chamber slot (25°C) and leave the cartridge in i-Chamber for 12 minutes.
   ⚠ *Scan the sample-loaded cartridge immediately when the incubation time is over. If not, it will cause inexact test result.*
6) To scan the sample-loaded cartridge, insert it into the cartridge holder of the instrument for ichroma™ tests. Ensure proper orientation of the cartridge before pushing it all the way inside the cartridge holder. An arrow has been marked on the cartridge especially for this purpose.
7) Tab 'START' button on the instrument for ichroma™ tests to start the scanning process.
8) Instrument for ichroma™ tests will start scanning the sample-loaded cartridge immediately.
9) Read the test result on the display screen of the instrument for ichroma™ tests.

(Please refer to the ichroma™ II operation manual for complete information and operation instructions.)

▶ **ichroma™-50**

1) Insert the tip array in the tip station.
2) Insert the detector array in the Reagent station and cover the reagent station.
3) Open the diluent and insert the diluent in the diluent station.
4) Open the cover of the magazine station and pull and lift the cartridge magazine.
5) Insert the cartridges in the cartridge magazine one by one.
6) Insert the cartridge loaded cartridge magazine into the magazine station and close the cover of the magazine station.
7) Insert the sample tube into the blood collection tube rack and load the blood collection tube rack into the sampling station (loading part).
8) Tap the button which is provided in the upper side of the No. of test cartridge region to select ID chip what you want to use.
9) When the selected cartridge slot is activated, set the number of test cartridge by tapping.
10) Tap the button which is provided in the upper side of the No. of reagent region to select ID chip what you want to use.
11) When the selected slot is activated, set the number of Detector by tapping.
12) Set the number of pipette tips by tapping.
13) Tap the 'START' button on the left upper of the main screen to start test.

(Please refer to the ichroma™-50 operation manual for complete information and operation instructions.)

INTERPRETATION OF TEST RESULT

- The instrument for ichroma™ tests calculates the test result automatically and displays NT-proBNP concentration of the test sample in terms of pg/mL.
- The cut-off (reference value): 300 pg/mL
- The working range of the **ichroma™ NT-proBNP** is 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.
- The control tests should be performed immediately after opening a new test lot to ensure the test performance is not altered.
- Quality control tests should also be performed whenever there is any question concerning the validity of the test results.
- Control materials are not provided with **ichroma™ NT-proBNP**. For more information regarding obtaining the control materials, contact Boditech Med Inc.'s Sales Division for assistance.
  (Please refer to the instruction for use of control material.)

PERFORMANCE CHARACTERISTICS

- **Analytical sensitivity**

Limit of Blank (LOB)	6 pg/mL
Limit of Detection (LOD)	10 pg/mL
Limit of Quantitation (LOQ)	30 pg/mL

- **Analytical specificity**
  - Cross-reactivity
    There was no significant cross-reactivity from these materials with the ichroma™ NT-proBNP test measurement.

Cross-reactivity material	Conc. (pg/mL)
Troponin Complex	1,000
CK-MB	1,000
D-Dimer	20,000
Myoglobin	10,000

- Interference
  There was no significant interference from these materials with the ichroma™ NT-proBNP test measurement.

Interference material	Conc.
Bilirubin	350 µmol/L
Cholesterol	13 mmol/L
D-Glucose	1,000 mg/dL
Hemoglobin	2 g/L
L-Ascorbic acid	350 µmol/L
Triglyceride mixture	500 mg/dL

- **Precision**

- Between lot
  One person tested three different lots of **ichroma™ NT-proBNP**, ten times at each concentration of the control standard.
- Between person
  Three different persons tested **ichroma™ NT-proBNP**; ten times at each concentration of the control standard.
- Between day
  One person tested **ichroma™ NT-proBNP** during five days; ten times at each concentration of the control standard.
- Between site
  One person tested **ichroma™ NT-proBNP** at three different sites; ten times at each concentration of the control standard.

Conc.	between Lot		between person	
(pg/mL)	mean	CV (%)	mean	CV (%)
234	233.43	5.93	233.98	5.55
1875	1844.81	6.32	1870.93	5.65
15000	15060.43	5.34	14950.65	5.27
Conc.	between day		between site	
(pg/mL)	mean	CV (%)	mean	CV (%)
234	234.76	6.35	234.10	6.26
1875	1866.67	5.90	1884.38	5.53
15000	14894.60	6.22	14973.26	5.88

- **Accuracy**
  The accuracy was confirmed by testing with three different lots of **ichroma™ NT-proBNP**. The tests are repeated 10 times in each different concentration.

Mean of 3 Lots						
Conc. [pg/mL]	Lot1	Lot2	Lot3	AVG	CV (%)	Recovery (%)
234	231.26	240.59	233.33	235.06	6.3	100.5
1875	1872.24	1860.88	1878.36	1870.49	6.3	99.8
15000	15016.80	14483.25	14786.10	14762.05	5.5	98.4

- **Comparability**
  NT-proBNP concentration of 100 clinical samples were independently with **ichroma™ NT-proBNP** and Cobas e411 (Roche Diagnostics Inc. Switzerland) as per prescribed test procedures. Test results were compared and their comparability was investigated with linear regression and coefficient of correlation (R). Linear regression and coefficient of correlation between the two tests were Y=0.9267X – 74.988 and R = 0.9906 respectively.

REFERENCES

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6. Hogenhuis J, Voors AA, Jaarsma T, Hoes AW, Hillege HL, Kragten JA, van Veldhuisen DJ. Anaemia and renal dysfunction are independently associated with BNP and NT-proBNP levels in patients with heart failure. Eur J Heart Fail. 2007 Aug;9(8):787-94. Epub 2007 May 25.
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Note: Please refer to the table below to identify various symbols

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

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