Instruction For Use

【Product Name】

N-terminal pro B-type Natriuretic Peptide(Electrochemiluminescence Immunoassay)

【Package】

1×100T, 2×100T

【Intended Use】

Immunoassay for the in vitro quantitative determination of N-terminal pro B-type natriuretic peptide in human plasma.

Heart failure (HF) is complex clinical symptoms of lesion of myocardial structure and function, caused by myocardial infarction, cardiomyopathy, hypertension, inflammation and so on. The main manifestations are dyspnea, weakness and fluid retention, eventually leading to lowering the ventricular pumping and filling function. N-terminal pro B-type natriuretic peptide (NT-proBNP) and B-type natriuretic peptide (BNP) are recommended indicators of heart failure. The plasma levels of both BNP and NT-proBNP are markedly increased in subjects with left ventricular dysfunction, and correlate well with the New York Heart Association functional classification of heart failure[1,2]. ProBNP is comprising 108 amino acids secreted mainly by the ventricle and then cleaving into physiologically active BNP (77-108) and the N-terminal fragment NT-proBNP (1-76)[3].

Determination of NT-proBNP levels in plasma can be useful in identifying patients with chronic heart failure, assessing the severity, predicting increased morbidity, and monitoring the therapeutic response[4,5,6].

【Test Principle】

Sandwich principle. Total duration of assay: 18 minutes

- 1st incubation: a sample, biotinylated monoclonal NT-proBNP-specific antibody and monoclonal NT-proBNP-specific antibody labeled with a ruthenium complex react to form a sandwich complex.
- 2nd incubation: After addition of streptavidin-coated microparticles, the complex binds to the solid phase via interaction of biotin and streptavidin.
- Measurement: The reaction mixture is aspirated into the measuring cell where the microparticles are magnetically captured onto the surface of the electrode. Unbound substances are then removed with Buffer. Application of a voltage to the electrode then induces chemiluminescent emission which is measured by a photomultiplier.
- Results are determined via calibration and a master curve provided via the reagent barcode.

【Main Component】

The reagent pack consists of MB, RA, RB and calibrators, different lots cannot be used at the same time or mixed up together.

Component	Ingredients	Volume (1×100T)	Volume (2×100T)
(MB)	Streptavidin-coated microparticles, 0.75 mg/mL: 0.1M PBS; 0.05% ProClin™ 300	1×5.0 mL	2×5.0 mL
(RB)	Anti-NT-proBNP-Ab~biotin, 1.5 mg/L: 0.1M PBS; 0.05% ProClin™ 300	1×8.0 mL	2×8.0 mL
(RA)	Anti-NT-proBNP-Ab~Ru(bpy)$^{2+}_3$, 1.5 mg/L: 0.1M PBS; 0.05% ProClin™ 300	1×8.0 mL	2×8.0 mL
Calibrator (High)	Calf serum, 0.05% ProClin™ 300; lyophilized	1×1.0 mL	1×1.0 mL
Calibrator (Low)	Calf serum, 0.05% ProClin™ 300; lyophilized	1×1.0 mL	1×1.0 mL

ProClin™ is a trademark of the Dow Chemical Company or an affiliated company of Dow.

The assignment of calibrators value complies strictly with ISO 17511:2003, and this method can be traced back to the Roche Elecsys proBNP assay (REF 04842464).

Materials and instruments required but not provided:

- Auffer
- Buffer
- Concentrated Washing Buffer
- eCL8000 series Automated ECL Immunoassay Analyzer
- Assay cup
- Multi Control Cardiac Marker

【Storage and Shelf Life】

Unopened reagent and calibrators should be placed at 2~8ºC and will be valid until expiration date.

Opened reagents should be used and stored at 2~8ºC in 28 days, otherwise trashed.

The calibrators are lyophilized, should be used as soon as possible after redissolved, or subpackaged into small tubes and refrigerated at -20ºC, whose period of validity is only 90 days.

The expiration date is labeled on the box, rackpack and bottles.

Damaged, expired or contaminated reagents should be discarded.

【Applicable Instrument】

Automated ECL Immunoassay Analyzer: eCL8000, eCL8000i, eCL8000p, eCL8000x.

【Specimen collection, handling and storage】

Human plasma added with heparin lithium, heparin sodium, EDTA-K_2 and EDTA-K_3 anti-coagulants are recommended. Blood samples should be collected by standard operation of venous puncture; after complete coagulation, the tangible component should be removed by centrifugation. The sample should avoid bubbling during testing. Lipid layer floating on the upper of the sample should be removed. Samples with severe hemolysis are not in suggestion. Samples would better to be tested in 4 hours after collection, otherwise, should be stored 2~8ºC for no more than 8 hours or -20ºC, 90 days. Freezing and thawing cycle is permitted only once.

Due to possible evaporation factors, samples, calibrators and controls on the analyzers should be analyzed/measured within 2 hours .

【Assay Procedure】

Testing procedures and precautions

Previous to operation, users should read the operational manual carefully to obtain system operation procedure, sample processing, security precaution, maintenance and other related information.

Set up N-terminal pro B-type natriuretic peptide (NT-proBNP) test according to the operational manual.

Put the NT-proBNP rackpack into the correct analyzer slot to automatically suspend magnetic beads at least 30 min before testing.

The sample volume required for each NT-proBNP test is 45 µL.

Calibration

Calibration should be performed using lot-matching reagent and calibrators.

Before calibration, the reagent and main curve information should be imported into the analyzer via radio frequency identification (RFID) reagent card(refer to instrument user manual). The analyzer adjusts the main curve to produce working curve according to the calibrator results, and identifies validity of the working curve automatically.

Recalibration is recommended when:

(1) the reagents of different lot are used;

(2) the same lot of reagents were used on the analyzer beyond 28 days;

(3) quality control misses the target.

Calibrator handling :

Carefully dissolve the contents of one bottle by adding exactly 1.0 mL deionized water and allow to stand 10~20 minutes to reconstitute. Mix fully, avoiding foam formation. Validity of the reconstituted calibrators, sustains either at 2~8ºC for 7 days or at -20ºC for 3 months.

It is recommended to aliquot the calibrators after reconstitution and store at -20ºC for later use.

Quality control

In order to ensure the reliability of test results, it's recommended to test the control materials every 24 hours. After each calibration, reagent lot change, maintenance or failure repairment, quality control is recommended. The quality control results should fall within the scope of local regulations. If beyond, the analyzer status, reagents, calibration and other factors should be checked.

This kit do not contain control materials. It is recommended to use the Multi Control Cardiac Marker of Shenzhen Lifotronic Technology Co., Ltd.

Calculation

The system software automatically calculates the analyte concentration using particular algorithm, and the result unit is pg/mL or pmol/L: pg/mL × 0.118 = pmol/L, pmol/L × 8.457 = pg/mL.

Specimen dilution

Samples exceeding the measuring range would be manually diluted with NT-proBNP-negative plasma.

The recommended dilution ratio is 1:2, and the diluted sample test results need to multiply dilution ratio.

【Biological reference interval】

According to plasma test results of 973 asymptomatic human in Guangdong and Zhejiang Province, P. R. China.The data are shown in the following table:

Age	<75 years	≥75 years
N	698	275
Mean (pg/mL)	24.04	399.68
95th percentile (pg/mL)	125.2	521.6

Due to differences in the region, race, gender and age, laboratories are recommended to set up their own reference ranges.

【Result Interpretation】

For explaining the result, the patient's overall clinical manifestation should be referred to, including symptoms, medical history and other relevant data and information.

【Limitations】

Test results are used only for clinical reference and cannot be used as the basis for diagnosis or rule-out of diseases alone.

Even the concentration of NT-proBNP reached 100000 pg/mL, hook effect is unobservable.

When the sample containing bilirubin ≤ 25 mg/dL, hemoglobin ≤500mg/dL, lipid ≤ 2000 mg/dL, biotin ≤ 20 ng/mL, or HAMA (human anti-murine antibodies) ≤ 100 ng/mL, interference deviation to the test result lies within ±10%. The test result would not be interfered by rheumatoid factor (1100 IU/mL).

【Measuring Range】

The measuring range of the kit is 5 ~ 35000 pg/mL. For samples with NT-proBNP content below the lower detection limit, the results are reported in <5 pg/mL; If the NT-proBNP content is above the upper detection limit, then the sample should be diluted and re-tested. The results are reported after multiplying with the dilution factor.

【Analytical Performance】

Lower limit of detection

The lower detection limit is 5 pg/mL .

Precision

The relative bias of measuring different concentration of the NT-proBNP antigen additive samples should fall between 0.90 and 1.10.

Linearity

The correlation coefficient (Pearson's r) is not less than 0.9500 in the interval of 5 ~ 35000 pg/mL.

Within-run precision (repeatability)

The coefficient of variation (CV) is less than 8%.

Between-lot precision

The coefficient of variation (CV) is less than 10%.

Analytical specificity

Test results of blank samples spiked with analogs below are lower than 5 pg/mL.

Analog	Concentration (pg/mL)
Angiotensin II	600
Angiotensin III	1000

【Method Comparison】

Measuring fresh clinical samples with Lifotronic NT-proBNP assay and Roche proBNP II assay, respectively, two results gave linear correlation.

Regression equation: y = 0.9436x+39.906, Pearson's r = 0.9717 (concentration range: 48.4~10130 pg/mL).

【Precaution and Warning】

The kit is only used for in vitro diagnostics.

When using the kit, it's necessary to comply with regulations in the laboratory.

All reagents and samples including specimen, calibrators and control materials, should avoid foaming before and during test.

Test results of the kit are for clinical reference only, and clinical evaluation of patients should be combined with their symptoms and signs, medical history, other laboratory examination results and treatment responses.

Due to factors such as methodology or antibody specificity, testing identical samples with reagents from different manufacturers may get different results, and the results from different kits should not compare directly, lest cause the wrong medical explanation; It's suggested that laboratorians should point out the characteristics of used reagent in the test report to the clinician. In serial monitoring, if the reagent type is changed, a continuous parallel test should be performed and comparison of the results to the former to determine new baseline value.

This product contains animal-sourced materials and may have potential biological risk. All samples and test wastes should be treated as the source of infection, and all wastes must be disposed according to local regulations.

The preservative ProClin™ 300 contains 3% 2-methyl-4-isothiazolin-3-one (MIT) and 5-chloro-2-methyl-4-isothiazolin-3-one (CMIT), will be harmful if inhalation, contact with skin and/or swallow, and may be toxic to aquatic organisms; thus, proper personal protection should be adopt while handling the reagent, and abandoned reagents should be dealed with in compliance with local regulations.

【Symbol】

Symbol	Title of Symbol
	Manufacturer
	Authorized representative in the European Community
	Use by
	Lot number
	Serial number
	Temperature limitation
	Consult instructions for use
	In vitro diagnostic medical device
	Indicates this device is in compliance with Europe Directive.
	Sufficient for <n> tests
	Biological risks
	This way up

【Reference】

1. Hunt PJ, *et al*. Immunoreactive aminoterminal pro-brain natriuretic peptide (NT-proBNP): a new marker for cardiac impairment. Clin Endocrinol 1997;47:287-96.
2. Talwar S, *et al*. Plasma N-terminal pro-brain natriuretic peptide and the ECG in the assessment of left ventricular systolic dysfunction in a high risk population. Eur Heart J 1999;20:1736-44.
3. Valli N, Gobinet A, Bordenave L. Review of 10 years of the clinical use of brain natriuretic peptide in cardiology. J Lab Cin Med 1999;134:437-444.
4. Cardarelli R, *et al*.B-type Natriuretic Peptide: a review of its diagnostic,prognostic,and therapeutic monitoring value in heart failure for primary care physicians. J Am Board Fam Pract.2003,16(4):327-33.
5. McCullough PA, *et al*. Sorting out the evidence on natriuretic peptides. Rev Cardiovasc Med 2003;4:S13-S19.
6. Gardner RS, *et al*. N-terminal pro-brain natriuretic peptide, a new gold standard in predicting mortality in patients with advanced heart failure. Eur Heart J 2003;24:1735-43.

【Manufacturer】

Shenzhen Lifotronic Technology Co., Ltd.

Unit A, 4th Floor, Building 15, Yijing Estate, No.1008 Songbai Road, Nanshan District, Shenzhen City, Guangdong Province, 518055, P.R.China

E-mail: Inter-service@lifotronic.com

【European Representative】

Shanghai International Holding Corp. GmbH (Europe)

Eiffestrasse 80, 20537 Hamburg, Germany

Tel.+49-40-2513175 Fax.+49-40-255726

【Version and Revision】

Version: A0

Revision Date: Aug. 9th, 2018 P/N:H-07-R05A101-00002 A0