Snibe Diagnostic

CE REF 130206004M: 100 tests
130606004M: 50 tests

MAGLUMI® NT-proBNP (CLIA)

INTENDED USE

The kit is an *in vitro* chemiluminescence immunoassay for the quantitative determination of N-terminal prohormone b-type natriuretic peptide (NT-proBNP) in human serum with the MAGLUMI series Fully-auto chemiluminescence immunoassay analyzer (including Maglumi 600, Maglumi 800, Maglumi 1000, Maglumi 1000 Plus, Maglumi 2000, Maglumi 2000 Plus, Maglumi 4000, Maglumi 4000 Plus, MAGLUMI X8, MAGLUMI X3 and MAGLUMI X6) and Biolumi series Integrated System (including Biolumi CX8).

SUMMARY AND EXPLANATION OF THE TEST

Brain natriuretic peptide or B-type natriuretic peptide (BNP) (also ventricular natriuretic peptide or natriuretic peptide B) is a 32-amino acid polypeptide secreted by the ventricles of the heart in response to excessive stretching of heart muscle cells (cardiomyocytes). BNP is synthesized as a 134-amino acid preprohormone (preproBNP), encoded by the human gene NPPB. Removal of the 25-residue N-terminal signal peptide generates the prohormone, proBNP, which is stored intracellularly as an O-linked glycoprotein; proBNP is subsequently cleaved between arginine-102 and serine-103 by a specific convertase (probably furin or corin) into NT-proBNP and the biologically active 32-amino acid polypeptide BNP-32, which are secreted into the blood in equimolar amounts[1-3].

B-type natriuretic peptide (BNP) and the amino-terminal fragment of the BNP prohormone (NT-proBNP) are markers for functional cardiac impairment and are elevated in heart failure (HF)[4-5]. Assays for the cardiac peptides atrial natriureti c peptide (ANP) and B-type natriuretic peptide (BNP) have received considerable attention as potential screening tests for symptomatic and asymptomatic heart disease .These looped peptides and the amino-terminal fragments of their precursor hormones (NT-proANP and NT-proBNP) are secreted by the hemodynamically stressed heart, mainly in response to myocardial stretch induced by volume overload[6]. Both ANP and BNP promote natriuresis and diuresis, inhibit the renin –angiotensin –aldosterone axis and act as vasodilators[7]. In contrast to ANP, regulation of BNP synthesis and excretion occurs mainly at the level of gene expression, suggesting that ANP and BNP form a dual, integrated natriuretic peptide system, and BNP may be a backup hormone activated only after prolonged ventricular overload[8].

Available clinical data indicate that both BNP and NT-proBNP appear to be useful diagnostic and prognostic markers with respect to heart failure (HF) and may be superior to ANP and NT-proANP[4]. The main clinical utility of NT-proBNP is that a normal level rules out acute heart failure in the emergency setting. An elevated NT-proBNP should never be used to "rule in" acute or chronic heart failure in the emergency setting due to lack of specificity. NT-proBNP can also be used for screening and prognosis of heart failure. NT-proBNP are also typically increased in patients with left ventricular dysfunction, with or without symptoms[9-11].

PRINCIPLE OF THE TEST

The NT-proBNP assay is a sandwich chemiluminescence immunoassay.

Use ABEI to label an anti-NT-proBNP monoclonal antibody, and use another monoclonal antibody to coat magnetic microbeads. These two monoclonal antibodies are obtained in natural NT-ProBNP immunogen. The binding sites are 1-9 and 61-76 area of NT-ProBNP amino acids. The sample (or calibrator/control, if applicable), ABEI Label and magnetic microbeads are mixed thoroughly and incubated, forming sandwich complexes; after precipitation in a magnetic field, decant the supernatant, then perform a wash cycle. Subsequently, the Starter 1+2 are added to initiate a flash chemiluminescent reaction. The light signal is measured by a photomultiplier as relative light units (RLUs), which is proportional to the concentration of NT-proBNP present in the sample (or calibrator/control, if applicable).

KIT COMPONENTS

Material Provided

Components	Contents	100 tests (REF:130206004M)	50 tests (REF: 130606004M)
Magnetic Microbeads	Magnetic microbeads coated with anti- NT-proBNP monoclonal antibody, containing BSA, NaN_3 (<0.1%).	2.5 mL	2.0 mL
Calibrator Low	Containing BSA and NT-proBNP antigen, NaN_3 (<0.1%).	3.0 mL	2.0 mL
Calibrator High	Containing BSA and NT-proBNP antigen, NaN_3 (<0.1%).	3.0 mL	2.0 mL
Buffer	Containing BSA, NaN_3 (<0.1%).	7.5 mL	4.5 mL
ABEI Label	Anti- NT-proBNP monoclonal antibody labeled with ABEI, containing BSA, NaN_3 (<0.1%).	12.5 mL	7.5 mL
Internal Quality Control 1	Containing BSA and NT-proBNP antigen, NaN_3 (<0.1%).	2.0 mL	2.0 mL
Internal Quality Control 2	Containing BSA and NT-proBNP antigen, NaN_3 (<0.1%).	2.0 mL	2.0 mL
All reagents are provided ready-to-use.			

Accessories Required But Not Provided

MAGLUMI and Biolumi Series:

Reaction Module	REF: 630003
Starter 1+2	REF: 130299004M, 130299027M
Wash Concentrate	REF: 130299005M
Light Check	REF: 130299006M
Reaction Cup	REF: 130105000101

Please order accessories from Shenzhen New Industries Biomedical Engineering Co., Ltd. (SNIBE) or our authorized representatives.

CALIBRATION

Traceability: This method has been standardized against the SNIBE internal reference substance.

Test of assay specific calibrators allows the RLU values to adjust the assigned master curve. Results are determined via a calibration curve which is instrument-specifically generated by 2-point calibration and a master curve (10 calibrations) provided via the reagent Radio Frequency Identification (RFID) CHIP.

Recalibration is recommended if any of the following conditions occurs:

- After each change of lots (Reagent or Starter 1+2).
- Every week and/or each time a new reagent kit is used (recommended).
- After instrument service is required.
- If controls lie outside the expected range.

QUALITY CONTROL

Follow government regulations or accreditation requirements for quality control frequency.
Internal quality control is only applicable with MAGLUMI and Biolumi systems. For instructions for use and target value refer to ***NT-proBNP (CLIA) Quality Control Information***. User needs to judge results with their own standards and knowledge.
For detailed information about entering quality control values, refer to the corresponding Analyzer Operating Instructions.
To monitor system performance and chart trends, commercially available quality control materials are required. Treat all quality control samples the same as patient samples. A satisfactory level of performance is achieved when analyte values obtained are within the acceptable Control Range for the system or within your range, as determined by an appropriate internal laboratory quality control scheme. If the quality control results do not fall within the Expected Values or within the laboratory's established values, do not report results. Take the following actions:

- Verify that the materials are not expired.
- Verify that required maintenance was performed.
- Verify that the assay was performed according to the instructions for use.
- Rerun the assay with fresh quality control samples.
- If necessary, contact your local technical supporters or distributors for assistance.

SPECIMEN COLLECTION AND PREPARATION

- Use standard sampling tubes or tubes containing separating gel. Collect blood aseptically following the universal precautions for venipuncture.
- Ensure that complete clot formation in serum specimens has taken place prior to centrifugation. Some specimens, especially those from patients receiving anticoagulant or thrombolytic therapy, may exhibit increased clotting time.
- If the specimen is centrifuged before a complete clotting, the presence of fibrin may cause erroneous results. Samples must be free of fibrin and other particulate matter.
- Do not use hemolyzed or grossly lipemic specimens as well as specimens containing particulate substance or exhibiting obvious microbial contamination. Inspect all specimens for bubbles, and remove bubbles before analysis for optimal results.
- Avoid repeating freeze-thaw cycles. The serum sample can be only frozen and thawed two times. Specimens must be mixed thoroughly after thawing.
- Centrifuged specimens with a lipid layer on the top must be transferred to a sample cup or a secondary tube. Care should be taken to transfer only the clarified specimen without the lipemic material.
- All samples (patient specimens and controls) should be tested within 3 hours when placed on board the MAGLUMI and Biolumi Systems. Refer to the SNIBE service for more details discussion of onboard sample storage constraints.
- Specimens removed from the separator, cells or clot may be stored up to 6 days at 2-8°C.
- Specimens can be stored up to 24 months frozen at -20°C or colder. Stored samples should be thoroughly mixed prior to use (Vortex mixer).
- Before shipping specimens, it is recommended that specimens be removed from the serum separator, red blood cells or clot. When shipped, specimens should be packaged and labeled in compliance with applicable state, federal and international regulations covering the transport of clinical specimens and infectious substances. Specimens should be shipped frozen.
- The sample volume required for a single determination of NT-proBNP is 100 µL.

WARNING AND PRECAUTIONS FOR USERS

- IVD
- For *In Vitro* Diagnostic Use.
- Follow the package insert carefully. Reliability of assay results cannot be guaranteed if there are any deviations from the instructions in this package insert.

Safety Precautions

- **CAUTION**: This product requires the handling of human specimens. It is recommended that all human sourced materials be considered potentially infectious and handled in accordance with the 29 CFR 1910.1030 Occupational exposure to bloodborne pathogens. Biosafety Level 2 or other appropriate biosafety practices should be used for materials that contain or are suspected of containing infectious agents.
- All samples, biological reagents and materials used in the assay should be considered potentially able to transmit infectious agents. They should therefore be disposed in accordance with the practices of your institution. Discard all materials in a safe and acceptable manner and in compliance with prevailing regulatory requirements.
- This product contains Sodium Azide. Dispose of contents and containers must be in accordance with all local, regional and national regulations.
- Refer to safety data sheets which are available on request.

Handling Precautions

- Do not use reagent kits beyond the expiration date.
- Do not interchange reagent components from different reagents or lots.
- Prior to loading the reagent kit on the system for the first time, the reagent kit requires mixing to re-suspend magnetic microbeads that have settled during shipment.
- For magnetic microbeads mixing instructions, refer to the Preparation of the Reagent section of this package insert.
- To avoid contamination, wear clean gloves when operating with a reagent kit and samples.
- Over time, residual liquids may dry on the septum surface. These are typically dried salts which have no effect on assay efficacy.
- For detailed discussion of handling precautions during system operation, refer to the SNIBE service information.

STORAGE AND STABILITY

- Sealed: Stored at 2-8°C until the expiration date.
- Opened at 2-8°C: Minimum stability is 4 weeks.
- On-board: Minimum stability is 4 weeks.
- To ensure the best kit performance, it is recommended to place opened kits in the refrigerator after the end of the intraday test work.
- Keep upright for storage to facilitate later proper resuspension of magnetic microbeads.
- Keep away from sunlight.

TEST PROCEDURE

Preparation of the Reagent

- Resuspension of the magnetic microbeads takes place automatically when the kit is loaded successfully, ensuring the magnetic microbeads are totally resuspended homogenous prior to use.
- To ensure proper test performance, strictly adhere to the corresponding Analyzer Operating Instructions. Each test parameter is identified via a RFID CHIP on the Reagent kit. For further information please refer to the corresponding Analyzer Operating Instructions.

DILUTION

Sample dilution by analyzer is not available in this reagent kit.
Samples with concentrations above the measuring range can be diluted manually. After manual dilution, multiply the result by the dilution factor. Please choose applicable diluents or ask SNIBE for advice before manual dilution.

High-Dose Hook

No high-dose hook effect was seen for NT-proBNP concentrations up to 100,000 pg/mL.

LIMITATIONS

- A skillful technique and strict adherence to the instructions are necessary to obtain reliable results.
- Bacterial contamination or heat inactivation of the specimens may affect the test results.
- A result within the expected range does not rule out the presence of disease and should be interpreted together with other diagnostic procedures.
- Test results are reported quantitatively. However, diagnosis of a disease should not be based on the result of a single test, but should be determined in conjunction with clinical findings in association with medical judgement.
- Any therapeutical decision should also be taken on a case-by-case basis.
- Patient samples containing human anti-mouse antibodies (HAMA) may give falsely elevated or decreased values. Although HAMA-neutralizing agents are added, extremely high HAMA serum concentrations may occasionally influence results.

RESULTS

Calculation of Results

The analyzer automatically calculates the NT-proBNP concentration of each sample by means of a calibration curve which is generated by a 2-point calibration master curve procedure. The results are reported in the unit of pg/mL. For further information please refer to the corresponding Analyzer Operating Instructions.

Interpretation of Results

Concentration of NT-proBNP (pg/mL) for adults:

Age(years)	N	Average	Value of 95% distribution
18-44	486	34.8	96
45-54	286	48.9	123
55-64	275	88.6	225
65-74	86	117	325
≥75	24	241	610

Concentration of NT-proBNP (pg/mL) for children between 1 to 18 years:

Age (years)	N	Value of 75% distribution
1-3	12	234
4-6	23	115
7-9	31	93
10	10	71
11	52	92
12	20	94
13	25	116
14	16	65
15	23	72
16	28	83
17	26	70
18	13	52

Results may differ between laboratories due to variations in population and test method. If necessary, each laboratory should establish its own reference range.

PERFORMANCE CHARACTERISTICS

Precision

Precision for the NT-proBNP assay was determined as described in the CLSI EP5-A2. 4 human serum pools and 3 controls containing different concentration of analyte were assayed in duplicate at two independent runs per day for 20 testing days. The results are summarized in the following table:

Sample	Mean(pg/mL) (N=80)	Within-Run		Between-Run		Total	
		SD(pg/mL)	%CV	SD(pg/mL)	%CV	SD(pg/mL)	%CV
Serum Pool 1	45.834	2.611	5.70	0.780	1.70	2.725	5.95
Serum Pool 2	198.570	7.140	3.60	5.428	2.73	8.969	4.52
Serum Pool 3	2491.982	68.785	2.76	12.671	0.51	70.003	2.81
Serum Pool 4	30963.581	612.941	1.98	75.897	0.25	617.623	1.99
Control 1	64.310	3.446	5.36	0.918	1.43	3.566	5.55
Control 2	248.775	11.079	4.45	3.058	1.23	11.744	4.72
Control 3	3150.879	58.892	1.87	28.481	0.90	65.418	2.08

Limit of Blank (LoB)

The LoB for the NT-proBNP assay is 2.0 pg/mL.

Limit of Detection (LoD)
The LoD for the NT-proBNP assay is 5.0 pg/mL.

Measuring Range
2.0-35000 pg/mL (defined by the limit of blank and the maximum of the master curve). Values below the limit of blank are reported as <2.0 pg/mL. Values above the measuring range are reported as >35000 pg/mL.

Linearity
The assay is linear between 5.0 pg/mL and 35000 pg/mL based on a study performed with guidance from CLSI EP6-A. Nine equally distributed levels of samples were prepared by blending a serum sample containing NT-proBNP 38500 pg/mL with a serum sample depleted of NT-proBNP (0.0 pg/mL).The mean sample recovery ranged between 90% to 110%.

Method Comparison
A total of 132 samples in the range of 5.783 to 33476.841 pg/mL were tested using the NT-proBNP assay (y) and a commercially available immunoassay (x). The data from the resulting linear regressions are summarized as: $y=0.956x+7.6877$, $r^2=0.983$.

Analytical Specificity
The specificity of the assay was obtained by adding ProBNP (500 pg/mL), Angiotensine I (500 pg/mL), Angiotensine II (500 pg/mL) and Renin (500 pg/mL) to two serum samples at the indicated concentrations. No interference was found.

Endogenous Interference
Substances up to the following concentrations did not interfere with the assay:
- Bilirubin 25 mg/dL
- Hemoglobin 1000 mg/dL
- Triglyceride 1500 mg/dL

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SYMBOLS EXPLANATIONS

	Consult instructions for use		Manufacturer
2°C 8°C	Temperature limit (Store at 2-8 °C)		Use-by date
Σ	Contains sufficient for		Keep away from sunlight
	This way up	EC REP	Authorized representative in the European Community
IVD	*In vitro* diagnostic medical device	CONTENTS	Kit components
REF	Catalogue number	LOT	Batch code