Snibe Diagnostic

CE REF 130206016M: 100 tests
130606016M: 50 tests

MAGLUMI™ BNP (CLIA)

INTENDED USE

The kit is an *in vitro* chemiluminescence immunoassay for the quantitative determination of BNP in human EDTA plasma with the MAGLUMI Fully-auto chemiluminescence immunoassay analyzer (including Maglumi 600, Maglumi 800, Maglumi 1000, Maglumi 2000, Maglumi 2000 Plus, Maglumi 4000, Maglumi 4000 Plus and MAGLUMI X8).

SUMMARY AND EXPLANATION OF THE TEST

Natriuretic peptides are a family of structurally similar peptide hormones with the function of natriuresis, promoting vasodilation and inhibiting renin [1, 2]. BNP exists widely in brain, heart, lung, digestive tract and so on. Among them, the heart content is the highest, and the synthesis and secretion of BNP in the heart is mainly in the ventricle. BNP is a 32 amino acid peptide with a central loop containing 17 amino-acids linked by a disulfide bond between 2 cysteine residues. The specific circular structure of BNP makes it physiologically active, and the half-life of BNP is about 20 min[3]. BNP is derived from a brain natriuretic peptide precursor (proBNP), which cleave into two polypeptides, a N-terminal pro-brain natriuretic peptide comprising 76 amino acids(NT-proBNP) and a C-terminal peptide comprising 32 amino acids(BNP). Both of them are excreted into circulating blood [4]. The increased level of BNP is mainly impacted by left ventricle-wall tension and blood load [5].

BNP measurement is mainly used in the clinical diagnosis, monitoring and prognosis of heart failure [6]. It has already been an important diagnostic criterion. The circulating level of BNP reflects the extent of ventricle volume extension, ventricle overload, and heart damage. BNP circulating levels increase as left ventricular function declines and clinical worsening symptoms of heart failure. BNP levels can be used to assess the severity of CHF, as they correlate with both New York Heart Association (NYHA) functional class and patient prognosis[7, 8, 9]. Besides, the determination of BNP has certain clinical diagnostic significance in myocardial infarction and acute coronary syndrome (ACS) [10, 11].

PRINCIPLE OF THE TEST

The BNP assay is a sandwich chemiluminescence immunoassay.

The sample (or calibrator/control, if applicable), ABEI labeled with anti-BNP monoclonal antibody and magnetic microbeads coated with anti-BNP monoclonal antibody 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 chemiluminescence reaction. The light signal is measured by a photomultiplier as relative light units (RLUs) which is proportional to the concentration of BNP present in the sample (or calibrator/control, if applicable).

KIT COMPONENTS

Material provided

Components	Contents	100 tests (REF:130206016M)	50 tests (REF:130606016M)
Magnetic Microbeads	Magnetic Microbeads coated with anti-BNP monoclonal antibody, containing BSA, NaN_3 (<0.1%).	2.5 mL	2.0 mL
Calibrator Low	Containing proBNP protein, BSA, NaN_3 (<0.1%).	2.0 mL	1.5 mL
Calibrator High	Containing proBNP protein, BSA, NaN_3 (<0.1%).	2.0 mL	1.5 mL
ABEI Label	Anti-BNP monoclonal antibody labeled with ABEI, containing BSA, NaN_3 (<0.1%).	12.5 mL	7.5 mL
Diluent	Containing BSA, NaN_3 (<0.1%).	10.0 mL	5.5 mL
Control 1	Containing proBNP protein, BSA, NaN_3 (<0.1%).	2.0 mL	1.5 mL
Control 2	Containing proBNP protein, BSA, NaN_3 (<0.1%).	2.0 mL	1.5 mL

Accessories Required But Not Provided

MAGLUMI Series:

Reaction Module	REF: 630003
Starter 1+2	REF: 130299004M, 130299012M, 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 system. For instructions for use and target value refer to ***BNP (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 operating instructions of MAGLUMI Fully-auto chemiluminescence immunoassay analyzer.
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

- Plasma collected using EDTA anticoagulate into plastic test tube can be applied to BNP assays[12, 13]. Other sample types, including serum, heparinized plasma, and citrated plasma samples provide lower BNP values, and are therefore not recommended.
- Collect blood aseptically following the universal precautions for venipuncture.
- Collect blood in plastic tubes containing EDTA as anticoagulant. All samples should be tested within 4 hours. If testing will be delayed for more than 4 hours, separate EDTA plasma from cells immediately after centrifugation, then store at 2-8°C or -20°C instantly. Specimens can be stored at 2-8°C for 24 hours and stored at -20°C or colder for 6 months.
- Do not use hemolyzed or grossly lipemic specimens as well as specimens containing particulate matter or exhibiting obvious microbial contamination. Inspect all specimens for bubbles, and remove bubbles before analysis for optimal results.
- Avoid repeating freeze-thaw cycles. The specimens can be frozen and thawed for three times. Stored samples should be thoroughly mixed prior to use (Vortex mixer). Frozen specimens should be mixed THOROUGHLY after thawing by LOW speed vortexing.
- Centrifuged specimens with a lipid layer on the top should 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 2 hours when being placed on board the MAGLUMI System. Refer to the SNIBE service for more detailed discussion of onboard sample storage constraints.
- Before shipping specimens, it is recommended that specimens be removed from the red blood cells. 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 BNP 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 of 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 sample.
- 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 6 weeks.
- On-board: minimum stability is 4 weeks. After this period, it is still possible to keep on using the Reagent Kit if the controls are found within the expected ranges.
- To ensure the best performance, it is recommended to put the open kit at 2-8°C, if it's not going to be used on-board in the next 12 hours.
- 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 operating instructions of MAGLUMII series Fully-auto chemiluminescence immunoassay analyzer. Each test parameter is identified via a RFID CHIP on the Reagent. For further information please refer to the operating instructions of MAGLUMII series Fully-auto chemiluminescence immunoassay analyzer.

DILUTION

Samples with concentrations above the measuring range can be diluted.
After manual dilution, multiply the result by the dilution factor. After dilution by the analyzers, the analyzer software automatically takes the dilution into account when calculating the sample concentration. The recommended dilution is 1:4 (either automatically by analyzers or manually).
The automatic sample dilution is available after dilution settings are done in the MAGLUMI series Fully-auto chemiluminescence immunoassay analyzer user software. Please refer to the operating instructions of MAGLUMI series Fully-auto chemiluminescence immunoassay analyzer.

High-Dose Hook
For the BNP assay, no high dose hook effect was observed when samples containing BNP up to 100,000 pg/mL.

LIMITATION

- 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 the patient's clinical picture and other diagnostic procedures.
- At diagnosis, BNP values should be used as an aid to other test data and the results should be interpreted in conjunction with other clinical and laboratory data.
- 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 plasma concentrations may occasionally influence results.

RESULTS

Calculation of Results
The analyzer automatically calculates the concentration in each sample by means of a calibration curve which is generated by a 2-point calibration master curve procedure. The results are expressed in pg/mL. For further information please refer to the operating instructions of MAGLUMII series Fully-auto chemiluminescence immunoassay analyzer.

Interpretation of Results
The expected ranges for the BNP assay were obtained by testing 379 apparently healthy people in China, and gave the following expected value:
<100 pg/mL (95th percentile)
Results may differ between laboratories due to variations in population and test method. It is recommended that each laboratory should establish its own reference range.

PERFORMANCE CHARACTERISTICS

Precision
Precision for BNP assay was determined as described in the CLSI EP5-A2. 4 human plasma pools and 2 controls containing different concentration of analyte were assayed in duplicate at two independent runs per day for 20 testing days on one analyzer. The result is 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
Plasma Pool 1	94.857	6.064	6.39	1.389	1.46	6.221	6.56
Plasma Pool 2	494.406	21.947	4.44	10.812	2.19	24.466	4.95
Plasma Pool 3	1988.986	68.114	3.43	17.320	0.87	70.281	3.53
Plasma Pool 4	3502.100	61.638	1.76	17.185	0.49	63.989	1.83
Control 1	201.973	12.345	6.11	2.043	1.01	12.513	6.20
Control 2	1001.970	39.971	3.99	28.417	2.84	49.844	4.97

Limit of Blank (LoB)
The LoB for BNP assay is 2.00 pg/mL.

Limit of Detection (LoD)
The LoD for BNP assay is 4.00 pg/mL.

Measuring Range
2.00-5000 pg/mL(defined by the limit of blank and the maximum of the master curve).Values below the limit of blank are reported as <2.00 pg/mL. Values above the measuring range are reported as >5000 pg/mL.

Linearity
The assay is linear between 4.00-5000 pg/mL based on a study performed with guidance from CLSI EP6-A. Nine equally distributed levels of samples were prepared by blending a plasma sample containing BNP 5550 pg/mL with a plasma sample containing BNP 4.00 pg/mL. The mean sample recovery ranged between 90% to 110%.

Method Comparison
A total of 117 samples in the range of 2.220 to 4809.8 pg/mL were tested by BNP assay (y) and a commercially available immunoassay (x). The data from the resulting linear regressions are summarized as: y=1.0038x +1.1987, r^2= 0.9957.

Analytical Specificity
The specificity of the assay was obtained by adding ANP (1000 pg/mL), CNP (1000 pg/mL), NT-proBNP(1-76) (1000 pg/mL) Angiotensin I (600 pg/mL), Angiotensin II (600 pg/mL) and Angiotensin III (1000 pg/mL) to three plasma samples containing 100 pg/mL, 800 pg/mL and 1000 pg/mL of BNP, respectively. No interference was found.

Endogenous Interference
Substances up to the following concentrations did not interfere with the assay:

- Bilirubin 20 mg/dL
- Hemoglobin 500 mg/dL
- Triglyceride 2000 mg/dL
- ANA 5 (S/CO)
- RF 1500 IU/mL
- HAMA 30 ng/mL

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EC REP

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SYMBOLS EXPLANATIONS

	Consult instructions for use		Manufacturer
8 °C 2 °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