N-terminal Prohormone of Brain Natriuretic Peptide (CLIA) PACKAGE INSERT

IVD

INTENDED USE

N-terminal Prohormone of Brain Natriuretic Peptide (CLIA) is a chemiluminescence immunoassay test for the quantitative measurement of circulating N-terminal prohormone of brain natriuretic peptide (NT-proBNP) in human serum, plasma and whole blood，which is intended as an aid to diagnosis of heart failure.
The detection range of this assay is 15pg/mL~35000pg/mL.
The test must be performed only on the ACCRE series analyzers (including ACCRE 6, ACCRE 8, ACCRE 90, ACCRE 100, ACCRE 120 and other ACCRE series analyzers)

For *in vitro* diagnostic use only. For professional use only.

SUMMARY AND EXPLANATIONS

Human BNP gene is located on the distal short arm of chromosome 1 and expresses a pre-pro-brain natriuretic peptide (preProBNP) which is composed of 134 amino acids. PreproBNP (134 amino acids) is processed to a proBNP (108 amino acids) precursor by cleaving a 26 amino acid fragment. In the process of which the brain natriuretic peptide precursor is secreted or entered the blood circulation, the proteolytic enzyme is divided into a N-terminal prohormone of brain natriuretic peptide (NT-proBNP) composed of 76 amino acids and from 32 amino acids. Composed of biological activity brain natriuretic peptide(BNP).The reference value of NT-proBNP increases with age. NT-proBNP has a longer (120 min) half-life than BNP (21 min). BNP is cleared from the circulation, with a half-life (t½) of approximately 23 minutes, by specific cellular receptors and neutral endopeptidases. Researches have shown that when the heart is damaged, NT-proBNP has shown advantages over BNP as a better detection indicator.
Studies have shown that when the heart is damaged, the concentration of NT-proBNP in plasma is more sensitive than BNP, indicating that NT-proBNP is a better detection indicator[2]. NT-proBNP is mainly synthesized and secreted by ventricular cells, so the NT-proBNP level in the blood reflects the structure and function of the ventricular structure[3,4,5], and clinically mainly used for auxiliary diagnosis of heart failure[6,7].

ASSAY PRINCIPLES

The principle of N-terminal Prohormone of Brain Natriuretic Peptide (CLIA) hereinafter also referred to as the "NT-proBNP" combines a double-antibody sandwich immunoassay method with enzyme catalytic chemiluminescence detection (CLIA). The principle is described as following:
Reagents for the assay are ready-to-use and pre-dispensed in the sealed reagent cartridge. All the assay steps are performed automatically by the ACCRE analyzer.
The sample is manually pipetted into the sample well of reagent cartridge, then sample, NT-proBNP antibody labeled with alkaline phosphatase and NT-proBNP antibody coated magnetic particles are mixed thoroughly together, after incubating. The antigen in the sample and the antibody on the magnetic beads and the alkaline phosphatase labeled antibody form an antibody-antigen sandwich immunocomplex. After the reaction is completed, the magnetic field adsorbed the beads and removes unbound substances by cleaning. The substrate is added to trigger the chemiluminescent reaction, the result of chemiluminescent reaction is measured by photomultiplier tube (PMT) as relative light units (RLUs). The concentration of the substance to be detected in the sample is proportional to the intensity of the luminescence. At the end, the results are automatically calculated by the ACCRE analyzer in relation to the calibration curve stored.

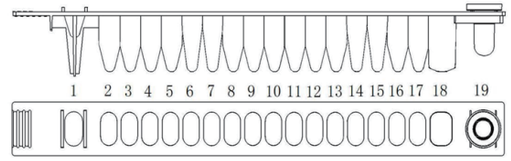
CONTENT OF THE KIT

Package: 60 tests/kit with quality controls, 36 tests/kit with quality controls, 60 tests/kit without quality controls, 36 tests/kit without quality controls
The calibrator in the kit has been traced to Roche's Brain natriuretic peptide precursor test kit (Electrochemical Luminescent Law) and its system. For specific calibrator concentration values, see the Calibrator Barcode Sheet for each lot.

Contents	Quantities				Notice
Manufacturers: Wondfo	REF W126A	REF W126C	REF W17A	REF W17C	
NT-proBNP Reagents Cartridges	60 strips	36 strips	60 strips	36 strips	Ready to Use
NT-proBNP Calibrator 1 (CAL1)	Dissolved to 1.0 mL x 1 vial	Dissolved to 1.0 mL x 1 vial	Dissolved to 1.0 mL x 1 vial	Dissolved to 1.0 mL x 1 vial	Lyophilized powder，Tris Buffer, 50mM; Proclin300, 0.48g/L; BND, 0.2g/L
NT-proBNP Calibrator 2 (CAL2)	Dissolved to 1.0 mL x 1 vial	Dissolved to 1.0 mL x 1 vial	Dissolved to 1.0 mL x 1 vial	Dissolved to 1.0 mL x 1 vial	Lyophilized powder, recombinant NT-proBNP, 125pg/mL
NT-proBNP Calibrator3 (CAL3)	Dissolved to 1.0 mL x 1 vial	Dissolved to 1.0 mL x 1 vial	Dissolved to 1.0 mL x 1 vial	Dissolved to 1.0 mL x 1 vial	Lyophilized powder, recombinant NT-proBNP, 2500pg/mL
NT-proBNP Quality Control Level 1 (QC1)	Dissolved to 1.0 mL x 1 vial	Dissolved to 1.0 mL x 1 vial	/	/	Lyophilized powder, recombinant NT-proBNP, 125pg/mL Only for the configuration with Quality Controls
NT-proBNP Quality Control Level 2(QC2)	Dissolved to 1.0 mL x 1 vial	Dissolved to 1.0 mL x 1 vial	/	/	Lyophilized powder，recombinant NT-proBNP, 5000pg/mL Only for the configuration with Quality Controls
Instruction for Use	1	1	1	1	N/A
Reagent Registration Card	1	1	1	1	N/A
Calibrator Barcode Sheet	1	1	1	1	N/A
Quality Control Sheet	1	1	/	/	Only for the configurations with Quality Control Sheet

Note: Refer to the target value list of the controls from Quality Control Sheet within the kit.

The Reagents Cartridge
The strip consists of 19 wells. The 1st well is the sample well and the last 19th is the reading well for chemiluminescent signal. From the 2nd to the 18th well are all covered with a label, aluminum foil seal. The label comprises a barcode which mainly indicates the reagent assay information, reagent lot number and expiration date. All reagents components needed for the assay are ready-to-use and pre-dispensed in the cartridge. And please do not interchange integral component from different reagents or lots.



Description of the reagent cartridge

Well	Component	Content	Volume
3	Sample treatment solution (R3)	Tris buffer, 50mmol/L; ProClin300, 0.48g/L; BND, 0.2g/L	50μL
5	Enzyme Label (R2)	ALP (alkaline phosphatase) labeled anti-NT-proBNP antibody (mouse), 2.0mg/L; MES Buffer, 50mmol/L； ProClin300, 0.48g/L; BND, 0.2g/L	50μL
7	Magnetic Particles (R1)	Anti-NT-proBNP antibody (mouse) coated magnetic particles, 0.2g/L; Tris buffer, 50mmol/L; ProClin300, 0.48g/L; BND, 0.2g/L	50μL
10, 11, 12, 14	Wash Solutions	Tris buffer, 50mmol/L; ProClin300, 0.48g/L; BND, 0.2g/L	350μL
16	Substrate Solution	Disodium [(4-chlorophenyl)sulfanyl] (10-methyl-9(10H)-acridinylidene) methyl phosphate	200μL

Note: Well 1, 2, 4, 6, 8, 9, 13, 15, 17, 18 are empty.

MATERIALS AND DISPOSABLES REQUIRED BUT NOT PROVIDED

- Sample diluent (manufactured by Tisenc)
- Pipette with disposable tip to dispense 200 μL.
- Powderless, disposable gloves.
- For other specific materials and disposables, please refer to the Instrument User Manual.
- Instruments of the ACCRE series analyzers.

WARNINGS AND PRECAUTIONS

- For *in vitro* diagnostic use only.
- For professional use only by qualified laboratory personnel.
- This kit contains products of animal origin. Certified knowledge of the origin and/or sanitary state of the animals does not totally guarantee the absence of transmissible pathogenic agents. It is therefore recommended that these products be treated as potentially infectious, and handled observing the usual safety precautions (do not ingest; do not inhale).
- Do not use reagents after the expiration date indicated on the box label.
- Do not use if the packaging of reagent cartridge is damaged or leaked.
- Do not use the reagents if the quantity is obviously insufficient.
- If any component adheres to inside wall of wells or aluminum film of the reagent cartridge, please gently switch the reagent cartridge to let the component back the wells.
- Do not reverse the reagent cartridge. Otherwise, the result will be unreliable.
- Do not mix reagents (or disposables) from different lots.
- Package insert instructions must be carefully followed. Reliability of assay results cannot be guaranteed if there are any deviations from the instructions in this package insert
- Use powderless gloves, as powder has been reported to cause false results for certain enzyme immunoassay tests.

STORAGE

- Store the N-terminal Prohormone of Brain Natriuretic Peptide (CLIA) kits including reagent cartridges, calibrators and quality controls **upright** at 2~8°C in dark place and the kit can be valid for 18 months from manufactured date.
- After the reagent cartridge is on-board in the ACCRE analyzer, the testing should be finished within 2 hours.
- When opened and re-dissolved, the calibrators and quality control will be stable for 45 days at -20°C and below -20°C. Calibrators and quality controls can only be frozen and thawed 3 times.
- Keep away from sunlight.
- Do not freeze reagent cartridges

SAMPLES

The sample volume for N-terminal Prohormone of Brain Natriuretic Peptide (CLIA) assay is 50μL, please note that at least 75μL of sample should be pipetted manually into the reagent cartridge.

Sample type and collection
Human serum or plasma/whole blood (Heparin lithium/EDTA-K_2) are recommended.
Types of tubes:
- Plastic tube with clot activator,
- Plastic tube with clot activator and separation gel,
- Plastic tube with Heparin lithium,
- Plastic tube with EDTA-K_2.

It is recommended to validate collection tubes before using them as some may contain substances which interfere with test results.
Note: Blood sampling tube results may vary from one manufacturer to another depending on the materials and additives used.
It is the responsibility of each laboratory to validate the type of sample tube used and to follow the manufacturer's recommendations for use.

Sample preparation
The current revision of the WHO/DIL/LAB/99.1 document provides recommendations for the preparation of samples.
For use of the sample tubes, follow the tube manufacturer's instructions for use.
The pre-analytical step, including the preparation of blood samples, is an essential part of medical analyses. In accordance with Good Laboratory Practice, this step is performed under the responsibility of the laboratory manager.
Insufficient clot time can result in the formation of fibrin with micro-clots that are invisible to the naked eye. The presence of fibrin, red blood cells, or suspended particles can lead to erroneous results.
Samples containing suspended fibrin particles or erythrocyte stroma should be centrifuged before testing.
If there are bubbles, precipitates or suspended solids in the sample, which may affect test results and should be removed by centrifugation before testing.
Samples with severe hemolysis (hemoglobin > 1000mg/dL) and heat inactivation are not recommended.
Preparation of frozen-stored samples: after thawing, these samples must be thoroughly mixed before testing.
Mix using with a vortex-type mixer. If necessary, clarify samples by centrifuging before testing.

Sample stability
Serum and plasma samples can be stored at 15~30°C for up to 4 hours or at 2-8°C for up to 6 days; if longer storage is required, freeze the serum or plasma at -20 °C for up to 3 months, without exceeding 3 freeze/thaw cycles.
Whole blood samples can be stored at 15~30°C for up to 4 hours or at 2-8°C for up to 24 hours and not undergone freeze/thaw cycles.

INSTRUCTIONS FOR USE

For complete instructions, see the Instrument User Manual.
When using the assay for the first time. Register the assay using the registration code in the reagent kit before any assay is used in the ACCRE analyzer for the first time. Calibration, QC and sample test are only available after assay was registered successfully.
When opening a new lot of reagents. Each new lot of reagent must be registered by using the registration code in Reagent Registration Card within the reagent kit.

Calibration
Calibration must be performed with the reagents and calibrators from Manufacturer. Each bottle of lyophilized calibrator should be re-dissolved by purified water to 1.0mL before use.
Calibration is necessary under the following conditions:
(1) New reagent lot number applied;
(2) Same lot of reagents used more than 4 weeks;
(3) Quality control out of control;
(4) When necessary, for example: system maintenance or repairment that may affect the analytical performance.

Test Procedure
1. Remove the kit from storage at 2-8 °C and take out the required number of reagent

cartridge. Allow the reagent cartridges, specimens, calibrators, and/or controls to equilibrate to room temperature (15-30°C) prior to testing.
2. Use each assay NT-proBNP strips for each sample, calibrator or control respectively.
3. Place the reagent cartridge into the reagent rack.
4. Manually pipette the Sample, Calibrator, or Controls into the sample well of reagent cartridge.

Please note that at least 75μL of sample has to be pipetted manually into the reagent cartridge and 50μL sample is needed for each test

** Before pipetting: Mix the samples using a vortex-type mixer if necessary, and ensure that the samples and sample diluent (if applicable) DO NOT have any bubbles. The same for calibrators and controls.*

5. Put the sample rack into the reagent chamber of ACCRE analyzer.
6. Place the assay tips into the reagent chamber of ACCRE analyzer.
7. Input Sample ID, sample type, dilution etc. into the software as directed by USER MANUAL.
8. N-terminal Prohormone of Brain Natriuretic Peptide (CLIA) assays will be completed within approximately 15 minutes. Assays will be performed automatically by the ACCRE analyzer.
9. After assay is completed, remove the reagent rack from the ACCRE analyzer. Dispose the used reagent cartridge into an appropriate recipient.

Dilution

Samples with concentrations above 35000pg/mL can be diluted with sample diluent manually. The recommended dilution is 1:2, And the concentration after dilution should be more than 17500pg/mL. After manual dilution, multiply the result by the dilution factor. ACCRE analyzer cannot perform automatic dilution for samples.

Quality control

Each bottle of lyophilized quality control should be re-dissolved by purified water to 1.0mL before use.

Quality control can be performed in accordance with local regulations or requirements related to accreditation, as well as requirements defined in the laboratory's quality control procedure.

RESULTS AND INTERPRETATION

Calculation of Results

The results are automatically calculated by the ACCRE analyzer using stored calibration curve which generate by 3-point calibration, and calculated according to a predefined mathematical model, the results expressed in pg/mL.

Interpretation of Results

The detection range is from 15pg/mL to 35000pg/mL. For samples with NT-proBNP below the upper limit of detection, quantitative determination can be performed. If the sample content is below the detection lower limit, the report result is <15pg/mL; if the sample content is higher than the detection limit, the report result is> 35000pg/mL.

Critical value

307 patients with stable heart failure were compared with 651 patients in the control group (healthy adults without heart disease). According to the receiver operating characteristic (ROC) curve (different NT-proBNP determination values correspond to clinical sensitivity and specificity), when the decision value is 125 pg/mL, the area under the curve is greater than 0.90. It shows good diagnostic performance, so the NT-proBNP project diagnoses a heart failure threshold of 125 pg/mL.

Serum samples from 651 healthy adults (18-90 years old) without heart disease in Guangdong were tested and analyzed. The test data were processed by non-parametric method. The data of this test is summarized in the following table:

Age（years）	18-44	45-54	55-64	65-74	≥75	Total
n	225	148	143	102	33	651
Average	36.1	48.7	72.2	107	210	66.8
The 95th percentile value (pg/mL)	97.50	119.1	215.4	312.3	610.1	192.9

Considering that the concentration of NT-proBNP in the blood is affected by age, serum samples from 374 minors (1-18 years old) in Guangdong were analyzed. The test data were processed by nonparametric method at 75th percentile:

Age (years)	1-3	4-6	7-9	10	11	12
n	18	25	34	16	82	24
The 75th percentile value (pg/mL)	233.3	116.1	97.01	71.00	90.02	95.00

Age (years)	13	14	15	16	17	18
n	30	24	30	34	35	22
The 75th percentile value (pg/mL)	125.3	62.00	74.59	84.67	68.07	50.82

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

LIMITATIONS

1. If the test results are inconsistent with clinical evidence, additional testing is suggested to confirm the result.
2. For diagnostic purposes, results should be used in conjunction with other data; e.g., symptoms, results of other tests, clinical impressions, etc.
3. Please note that heterophile antibody in human serum or plasma can interfere the test result.
4. High concentration of HAMA can also cause a false positive or false negative result.

PERFORMANCE CHARACTERISTICS

Detection limit

LOB (Limit of Blank) =10pg/mL.
LOD (Limit of Detection) =15pg/mL
LOB and LOD were determined according to CLSI® EP17-A2 recommendations.

Measurement range

The measurement range is the range of values corresponding to the acceptable performance limits (precision and linearity).

The measurement range of the N-terminal Prohormone of Brain Natriuretic Peptide (CLIA) assay is: 15 pg/mL~35000 pg/mL.

Linearity

The N-terminal Prohormone of Brain Natriuretic Peptide (NT-proBNP) kit is linear between 15pg/mL～35000pg/mL. The linear correlation coefficient(r) should be not less than 0.9900.

Precision

A precision study was performed according to CLSI® EP05-A3 recommendations. The within-lot precision CV% is less than 8%.
The between-lot precision CV% is less than 10%.

Accuracy

The sample which is a control with trueness traceability is used for the detection, and the relative deviation between the detection result and the calibration concentration is within ±10%.

Interfering Substances

Potential interference substances were studied according to CLSI ® EP07-A3 recommendations. No significant interference was detected up to the maximum concentrations tested.

drug	Drug concentration	Substance	Interference Concentration
Furosemide	≤60μg/mL	Hemoglobin	≤1000 mg/dL
Catopril	≤5μg/mL	Bilirubin	≤25 mg/dL
Losartan potassium	≤40μg/mL	Triglyceride	≤1500 mg/dL
Atenolol	≤10μg/mL	Total Protein	≤10 g/dL
Eplerenone	≤40μg/mL	Rheumatoid factor	≤1500 IU/mL
Valsartan	≤64μg/mL	/	/

Cross-reactivity

The N-terminal Prohormone of Brain Natriuretic Peptide (CLIA) assay is specific for N-terminal prohormone of brain natriuretic peptide. The following proteins were tested and found to have an insignificant effect on the measured NT-proBNP.

Substance	Concentration	Cross-reactivity
Angiotensin I	600 pg/mL	≤10 pg/mL
Angiotensin II	600 pg/mL	≤10 pg/mL
Angiotensin III	1000 pg/mL	≤10 pg/mL
ANP	3.1μg/mL	≤10 pg/mL
CNP	2.2 mg/mL	≤10 pg/mL
Aldosterone	0.6ng/mL	≤10 pg/mL
Renin	50ng/mL	≤10 pg/mL
Adrenomedullin	1ng/mL	≤10 pg/mL
Endothelin	20 pg/mL	≤10 pg/mL
Epinephrine	400pg/mL	≤10 pg/mL

Hook effect

No hook effect was found up to NT-proBNP concentrations of 300000pg/mL.

HAMA

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[12-15].

WASTE DISPOSAL

Dispose used or unused reagents as well as any other contaminated disposable materials following procedures for infectious or potentially infectious products.
It is the responsibility of each laboratory to handle waste and effluents produced according to their nature and degree of hazardousness and to treat and dispose of them (or have them treated and disposed of) in accordance with any applicable regulations.

INDEX OF SYMBOLS

Symbol	Meaning	Symbol	Meaning	Symbol	Meaning
	Consult instructions for use		Contains sufficient for <n> tests		Date of manufacture
	*In vitro* diagnostic medical device		Use-by date		Do not re-use
	Temperature limit		Batch code		Catalogue number
	Keep away from sunlight		Keep dry		Manufacturer
	Authorized representative in the European Community/ European Union		Do not use if package is damaged and consult instructions for use		Contains biological material of animal origin
	Biological risks		Caution		CE marking
	Upright		Fragile, handle with care		Do not roll
	Control				

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Shenzhen Tisenc Medical Devices Co., Ltd.
Address: 11F, 11G, Kechuang Building, Quanzhi Science and Innovation Park, Maozhoushan Industrial Park, Shajing Community, BaoAn District, Shenzhen, 518104, P.R.China
Phone: +86-0755-23225620
E-mail: globalmarketing@tisenc.com

EC REP

Shanghai International Holding Corp. GmbH (Europe)

Address: Eiffestrasse 80, 20537 Hamburg, Germany
Phone: +49-40-2513175
Fax: +49-40-255726
DIMDI No.: DE/0000040627
E-mail: shholding@hotmail.com

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