Snibe Diagnostic

CE REF 130206006M: 100 tests
130606006M: 50 tests

MAGLUMI® AⅡ (CLIA)

INTENDED USE

The kit is an *in vitro* chemiluminescence immunoassay for the quantitative determination of Angiotensin Ⅱ (AⅡ) in human plasma using 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

The renin–angiotensin system (RAS) or the renin–angiotensin–aldosterone system (RAAS) is a hormone system that is involved in the regulation of the plasma sodium concentration and arterial blood pressure[1]. Angiotensin Ⅰ is a peptide hormone that causes vasoconstriction and a subsequent increase in blood pressure. It is part of the renin-angiotensin system, which is a major target for drugs that raises blood pressure. Angiotensin also stimulates the release of aldosterone, another hormone, from the adrenal cortex. Aldosterone promotes sodium retention in the distal nephron, in the kidney, which also drives blood pressure up. It is derived from the precursor molecule angiotensinogen, a serum globulin produced in the liver[1-2]. Angiotensin Ⅱ (Ang Ⅱ) is an octapeptide hormone that plays an important role in cardiovascular homeostasis, which is converted from angiotensin Ⅰ through removal of two C-terminal residues by the enzyme angiotensin-converting enzyme (ACE), primarily through ACE within the lung (but also present in endothelial cells, kidney epithelial cells, and the brain)[1]. Typical of many proteins, Ang Ⅱ was named on the basis of its first-demonstrated biological function—the ability to act as a vasoactive agonist and induce contraction of blood vessels. Angiotensin Ⅱ is the biologically active product of the renin-angiotensin system. Angiotensin Ⅱ is very short-lived in the plasma: Once generated from angiotensin Ⅰ, it is degraded further into physiologically inactive peptides by various plasma peptidases, at a plasma half life of less than a minute[3-5].

Over the years, Ang Ⅱ has been shown to play important roles in mediating hypertension, heart failure, cardiac remodeling, diabetes, and the proliferative and inflammatory responses to arterial injury[6]. These findings have spawned the development of several classes of pharmacological agents designed at inhibiting the synthesis of Ang Ⅱ (eg, angiotensin Ⅱ-converting enzyme inhibitors) or blocking its action (eg, angiotensin Ⅱ receptor antagonists). These agents are now widely used in the treatment of hypertension and congestive heart failure[7].

PRINCIPLE OF THE TEST

The AⅡ assay is a competitive chemiluminescence immunoassay.

The sample, (or calibrator/control, if applicable), magnetic microbeads coated with anti-AⅡ polyclonal antibody are mixed thoroughly and incubated, forming antibody-antigen complexes. Then add ABEI labeled with purified AⅡ antigen, incubate to form complexes, after precipitation in a magnetic field, decant the supernatant, and 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 inversely proportional to the concentration of AⅡ present in the sample (or calibrator/control, if applicable).

KIT COMPONENTS

Material Provided

Components	Contents	100 tests (REF: 130206006M)	50 tests (REF: 130606006M)
Magnetic Microbeads	Coated with anti-AⅡ polyclonal antibody, containing BSA, NaN_3 (<0.1%).	2.5 mL	2.0 mL
Calibrator Low	Containing BSA and AⅡ antigen, NaN_3 (<0.1%).	3.0 mL	2.0 mL
Calibrator High	Containing BSA and AⅡ antigen, NaN_3 (<0.1%).	3.0 mL	2.0 mL
ABEI Label	AⅡ purified antigen labeled with ABEI, containing BSA, NaN_3 (<0.1%).	12.5 ml	7.5 ml
Internal Quality Control	Containing BSA and AⅡ antigen, NaN_3 (<0.1%).	2.0 mL	2.0 mL
Anticoagulant	0.3mol/L EDTA-2K buffer.	5.5 mL	5.5 mL
Enzyme Inhibitor	0.34mol/L 8-Quinolinol hemisulfate salt.	5.5 mL	5.5 mL
Dimercaprol	0.32 mol/L.	3.0 mL	3.0 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 ID/MS (isotope dilution mass spectrometry).

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 exchange 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 ***AⅡ (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

Specimen Collection

Collect blood aseptically following the universal precautions for venipuncture and then add the anticoagulants and enzyme inhibitors by the ratios below:

5 mL	Whole blood in one tube
+50 µL	0.30M EDTA-2K (If there're any precipitation of crystals, please use water bath to redissolve them before using.)
+25 µL	0.32 M Dimercaprol
+50 µL	Enzyme Inhibitors

Note:

- The anticoagulants and Enzyme Inhibitors could not be mixed together before added into the blood! (The correct procedure is anticoagulants firstly and the enzyme inhibitors secondly).
- If using the Vacuum tubes with anticoagulants additives, then please make sure that use EDTA (EDTA-2K or EDTA-4Na) as anticoagulant. In this situation, there is no need to add anticoagulant, only adding the Enzyme Inhibitor directly.
- If the volume of sample is different, please increase or decrease the reagents to the same proportion. For example, 2 ml blood, with 20 µL Anticoagulants, 10 µL Dimercaprol and 20 µL Enzyme Inhibitors.
- After sealing up the tube with cap, turn tube upside down several times to mix the sample. And place it in ice-water bath or 4°C refrigerator for 1-2 hours right now, then centrifuge the tube for 7 minutes with 4020 r/min, (preferably centrifuged at 4°C) to separate the plasma. If the plasma needs to be stored for long time, please seal and stored in the refrigerator (below -15°C).
- 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.
- Angiotensin Ⅱ could be converted to Angiotensin Ⅲ by angiotoninase A in the plasma, and also converted to Ang-(1-7) by prolyl Endopeptidase or PRCP. The specimen stored at 4°C or -20°C may be degraded, to ensure veracity in results, it is recommended that test is performed within 4 hours.
- Avoid repeating freeze-thaw cycles.
- 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 24 hours at 2-8°C.
- Specimens can be stored up to 30 days frozen at -20°C or colder.
- Before shipping specimens, it is recommended that specimens be removed from the plasma 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 AⅡ 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: Stable for 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.

LIMITATIONS

- As β-blockers, vasodilators, diuretics and steroids, licorice and other material interfere the renin, the PRA should be measured after stopping using them for two weeks. For reserpine and other drugs with slower metabolism, the PRA should be measured after three weeks. Patients who cannot stop using drug, can change to use drugs like guanethidine.
- PRA levels can be interfered by the intake of sodium, so the patient should decrease the intake of salt three days before the determination of PRA. Patients should be measured 24-hours-urinary sodium, before drawing blood and the results can be used for reference analysis.
- Provocation test: the patient does not get up in the morning, or lie down for two hours. Drawing blood between 6:00-8:00, and then inject furosemide with 0.7mg/kg body weight, but the total dose should less than 50mg. Keep upright for two hours (the patient can walk), then draw blood samples in excited state.
- During the two hours after injection of furosemide, a large amount of water and electrolytes were lost with the urine. If the patients have low serum potassium, it's better to supply that before drawing blood. Patients may feel thirsty, weakness, sweating, etc. during the test, generally not heavy. If the symptom is overweight, the test should be terminated.

RESULTS

Calculation of Results

The analyzer automatically calculates the AⅡ 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 corresponding Analyzer Operating Instructions.

Interpretation of Results

Reference values:

Diet	Status	Reference values (pg/mL)
Normal	Lying position	25-60
	Standing position	50-120

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 AⅡ assay was determined as described in the CLSI EP5-A2. 2 controls and 3 human plasma pools 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
Plasma Pool 1	25.385	1.220	4.81	1.457	5.74	1.900	7.48
Plasma Pool 2	60.065	3.214	5.35	2.394	3.99	4.008	6.67
Plasma Pool 3	120.290	3.576	2.97	3.034	2.52	4.690	3.90
Control 1	94.253	3.663	3.89	2.450	2.60	4.407	4.68
Control 2	249.701	7.147	2.86	5.465	2.19	8.997	3.60

Limit of Blank (LoB)

The LoB for the AⅡ assay is 5.0 pg/mL.

Limit of Detection (LoD)

The LoD for the AⅡ assay is 7.5 pg/mL.

Measuring Range

5.0-1000 pg/mL (defined by the limit of blank and the maximum of the master curve). Values below the limit of blank are reported as <5.0 pg/mL. Values above the measuring range are reported as >1000 pg/mL.

Recovery

Known concentrations of AⅡ were added to normal samples. The concentration of AⅡ was determined using the AⅡ assay, and the resulting percent recovery was calculated. The recovery should be within 90%-110%.

Sample	Added (pg/mL)	Observed (pg/mL)	%Recovery
	-	34.993	/
S1	11.23	46.571	103.10
	53.29	87.777	99.05
	-	92.333	/
S2	11.23	102.862	93.75
	53.29	148.315	105.05
	-	164.671	/
S3	11.23	175.761	98.75
	53.29	219.080	102.10

Method Comparison

A total of 100 clinical samples in the range of 25.88 to 738.03 pg/mL were tested using the AⅡ assay (y) and a commercially available immunoassay (x). The data from the resulting linear regressions are summarized as: y=0.969x+3.757, r^2=0.990.

Analytical Specificity

The specificity of the assay was obtained by adding AⅠ (10000 pg/mL) to plasma samples. No interference was found.

Endogenous Interference

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

- Bilirubin 12.5 mg/dL
- Hemoglobin 16 mg/dL
- Triglyceride 1250 mg/dL

REFERENCES

1. Peach, M. J. (1977). Renin-angiotensin system: biochemistry and mechanisms of action. Physiological reviews, 57(2), 313-370.
2. Weber, K. T., & Brilla, C. G. (1991). Pathological hypertrophy and cardiac interstitium. Fibrosis and renin-angiotensin-aldosterone system.*Circulation*,*83*(6), 1849-1865.
3. Oparil, S., & Haber, E. (1974). The renin-angiotensin system. *New England Journal of Medicine*, *291*(8), 389-401.
4. Ganong, W. F., & Ganong, W. (1995). *Review of medical physiology* (p. 59). Norwalk, CT: Appleton & Lange.
5. A. Leaf, GW Liddle (1974), in "Textbook of Endocrinology" p.938 ed. RH Williams, WB Saunders Co., Philadelphia.
6. Ruiz-Ortega, M., Lorenzo, O., Ruperez, M., Esteban, V., Suzuki, Y., Mezzano, S., & Egido, J. (2001). Role of the renin-angiotensin system in vascular diseases. *Hypertension*, *38*(6), 1382-1387.
7. Taubman, M. B. (2003). Angiotensin Ⅱ.

Shenzhen New Industries Biomedical Engineering Co., Ltd.
No.23, Jinxiu East Road, Pingshan District, 518122 Shenzhen, P.R. China
Tel: +86-755-21536601 Fax: +86-755-28292740

EC REP **Shanghai International Holding Corp. GmbH (Europe)**
Eiffestrasse 80, 20537 Hamburg, Germany
Tel: +49-40-2513175 Fax: +49-40-255726

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