Snibe Diagnostic

CE 0123 REF

130206511M:100 tests/kit
130606511M: 50 tests/kit
130706511M: 30 tests/kit

MAGLUMI® Direct Renin (CLIA)

INTENDED USE

The kit is an *in vitro* chemiluminescence immunoassay for the quantitative determination of Direct Renin in human plasma using the MAGLUMI series Fully-auto chemiluminescence immunoassay analyzer and Biolumi series Integrated System, and the assay is used for an aid in the diagnosis and treatment of a number of hypertension types in humans.

SUMMARY

Renin is a monomeric enzyme with an approximate M_w of 36000. It belongs to the aspartic acid protease family [1-3]. Renin is mainly synthesized in and released by the juxtaglomerular cells in the kidney [3]. Renin is found in the circulation in two forms. The form that catalyses the production of angiotensin I is sometimes referred to as 'active renin', but is often called 'plasma renin activity' or just simply 'renin'. The other form of renin found in plasma does not catalyse the production of angiotensin I, and is known as 'prorenin' [2]. All or almost all of the active renin its release is controlled by autonomic influences, stress changes, angiotensin II, sodium chloride and possibly other factors [3].

After release and activation, renin cleaves angiotensinogen produced by the liver into angiotensin I (inactive) which ultimately is processed by angiotensin converting enzyme (ACE) to the octapeptide angiotensin II. Angiotensin II binds to angiotensin II receptor (ATR) can cause vasoconstriction by stimulating the central nervous system; in addition it stimulates ADH (antidiuretic hormone) secretion and aldosterone secretion from the adrenal gland. When the concentration of Angiotensin II in the blood increases, it in turn inhibits the secretion of renin (negative feedback). The renin-angiotensin-aldosterone system (RAAS) plays a paramount role in water homeostasis and electrolyte balance, and in the regulation of arterial pressure [2,4,5]. Quantitative determination of Renin in human plasma samples in vitro is a useful tool to auxiliary diagnosis of a number of hypertension types in humans, such as essential hypertension, renal hypertension; then to take reasonable treatment plan. It's of great significance for monitoring the curative effect and prognosis evaluation [1,6-8].

TEST PRINCIPLE

Sandwich chemiluminescence immunoassay.

The sample, magnetic microbeads coated with anti-Renin monoclonal antibody, ABEI labeled with another anti-Renin monoclonal antibody are mixed thoroughly, reacting to form sandwich complexes and incubating. After precipitation in a magnetic field, the supernatant is decanted and then a wash cycle is performed. Subsequently, the Starter 1+2 are added to initiate a chemiluminescent reaction. The light signal is measured by a photomultiplier as relative light units (RLUs), which is proportional to the concentration of Renin present in the sample.

REAGENTS

Kit Contents

Component	Description	100 tests/kit	50 tests/kit	30 tests/kit
Magnetic Microbeads	Magnetic microbeads coated with anti-Renin monoclonal antibody (~4.00 μg/mL) in PBS buffer, NaN_3 (<0.1%).	2.5 mL	2.0 mL	1.0 mL
Calibrator Low	A low concentration of Renin antigen in PBS buffer, NaN_3 (<0.1%).	2.0 mL	1.0 mL	1.0 mL
Calibrator High	A high concentration of Renin antigen in PBS buffer, NaN_3 (<0.1%).	2.0 mL	1.0 mL	1.0 mL
ABEI Label	ABEI labeled with anti-Renin monoclonal antibody (~0.125 μg/mL) in Tris-HCl buffer, NaN_3 (<0.1%).	8.5 mL	5.5 mL	3.3 mL
Control 1	A low concentration of Renin antigen (40.0 μIU/mL) in PBS buffer, NaN_3 (<0.1%).	2.0 mL	1.0 mL	1.0 mL
Control 2	A high concentration of Renin antigen (105 μIU/mL) in PBS buffer, NaN_3 (<0.1%).	2.0 mL	1.0 mL	1.0 mL
All reagents are provided ready-to-use.				

The control barcode labels are provided.

Warnings and Precautions

- For *in vitro* diagnostic use.
- For professional use only.
- Exercise the normal precautions required for handling all laboratory reagents.
- Personal protective measures should be taken to prevent any part of the human body from contacting samples, reagents, and controls, and should comply with local operating requirements for the assay.
- A skillful technique and strict adherence to the package insert are necessary to obtain reliable results.
- Do not use kit beyond the expiration date indicated on the label.
- Do not interchange reagent components from different reagents or lots.
- Avoid foam formation in all reagents and sample types (specimens, calibrators and controls).
- All waste associated with biological samples, biological reagents and disposable materials used for the assay should be considered potentially infectious and should be disposed of in accordance with local guidelines.
- This product contains sodium azide. Sodium azide may react with lead or copper plumbing to form highly explosive metal azides. Immediately after disposal, flush with a large volume of water to prevent azide build-up. For additional information, see Safety Data Sheets available for professional user on request.

Note: If any serious incident has occurred in relation to the device, please report to Shenzhen New Industries Biomedical Engineering Co., Ltd. (Snibe) or our authorized representative and the competent authority of the Member State in which you are established.

Reagent Handling

- To avoid contamination, wear clean gloves when operating with a reagent kit and sample. When handling reagent kit, replace the gloves that have been in contact with samples, since introduction of samples will result in unreliable results.
- Do not use kit in malfunction conditions; e.g., the kit leaking at the sealing film or elsewhere, obviously turbid or precipitation is found in reagents (except for Magnetic Microbeads) or control value is out of the specified range repeatedly. When kit in malfunction conditions, please contact Snibe or our authorized distributor.
- To avoid evaporation of the liquid in the opened reagent kits in refrigerator, it is recommended that the opened reagent kits to be sealed with reagent seals contained within the packaging. The reagent seals are single use, and if more seals are needed, please contact Snibe or our authorized distributor.
- Over time, residual liquids may dry on the septum surface. These are typically dried salts and have no effect on assay efficacy.
- Use always the same analyzer for an opened reagent integral.
- For magnetic microbeads mixing instructions, refer to the Preparation of the Reagent section of this package insert.
- For further information about the reagent handing during system operation, please refer to Analyzer Operating Instructions.

Storage and Stability

- Do not freeze the integral reagents.
- Store the reagent kit upright to ensure complete availability of the magnetic microbeads.
- Protect from direct sunlight.

Stability of the Reagents	
Unopened at 2-8°C	until the stated expiration date
Opened at 2-8°C	6 weeks
On-board	4 weeks

Stability of Controls	
Unopened at 2-8°C	until the stated expiration date
Opened at 18-25°C	6 hours
Opened at 2-8°C	6 weeks
Frozen at -20°C	3 months
Frozen and thawed cycles	no more than 3 times

SPECIMEN COLLECTION AND PREPARATION

Specimen Types

Only the specimens listed below were tested and found acceptable.

Specimen Types	Collection Tubes
Plasma	K2-EDTA, Na2-EDTA

- The only sample material validated is human EDTA-plasma. Use of serum, heparinized plasma, and citrated plasma samples provides lower renin values, and is therefore not recommended.
- Careful standardization of the patient preparation and sampling conditions is strongly recommended. Collect blood at room temperature by venipuncture, in siliconized glass tubes, vacutainers (violet cap) or equivalent, containing EDTA as anticoagulant. The presence of haemolysis may indicate mistreating during sample collection or handling.
- The sample types listed were tested with a selection of sample collection tubes that were commercially available at the time of testing, i.e. not all available tubes of all manufacturers were tested. Sample collection systems from various manufacturers may contain differing materials which could affect the test results in some cases. Follow tube manufacturers' instructions carefully when using collection tubes.

Specimen Conditions

- When prorenin, the inactive precursor of renin, is cryoactivated to renin during sample handling, falsely elevated results are obtained. Cryoactivation occurs when patient samples are chilled to temperatures of 4°C or below for extended periods of time, and when samples are chilled but still liquid (i.e. not frozen). Cryoactivation of prorenin to renin occurs more rapidly in serum [9]. Prorenin blood concentration is approximately ten-fold greater than that of renin.
- Fasting specimens are recommended but not required. Record the time of day and the patient's posture during blood collection (supine, upright or seated).
- Do not pre-chill EDTA blood collection tubes nor store tubes on ice, but process blood at room temperature. Centrifuge tubes in a non-refrigerated centrifuge, separate EDTA-plasma from cells immediately after centrifugation, then aliquot and deep-freeze at -20°C or below instantly.
- Do not use heat-inactivated samples or grossly hemolyzed/hyperlipidaemia specimens and specimens with obvious microbial contamination.
- Samples must be free of fibrin and other particulate matter.
- To prevent cross contamination, use of disposable pipettes or pipette tips is recommended.

Preparation for Analysis

- It is recommended to test plasma samples immediately after loading on to the instrument.
- Carefully thaw before testing, mix the thawed samples and check for and remove foam with an applicator stick before analysis. Use a new applicator stick for each specimen to prevent cross contamination.
- Frozen specimens must be completely thawed before mixing. Mix thawed specimens thoroughly by low speed vortexing or by gently inverting. Visually inspect the specimens. If layering or stratification is observed, mix until specimens are visibly homogeneous. If specimens are not mixed thoroughly, inconsistent results may be obtained.
- Specimens should be free of fibrin, red blood cells, or other particulate matter. Such specimens may give reliable results and must be centrifuged prior to testing. Transfer clarified specimen to a sample cup or secondary tube for testing. For centrifuged specimens with a lipid layer, transfer only the clarified specimen and not the lipemic material.
- The sample volume required for a single determination of this assay is 100 μL.

Specimen Storage

Specimens removed from the separator, red blood cells or clot may be stored up to 4 hours at 18-25°C, or stored up to 2 months frozen at -20°C. Frozen specimens subjected to up to 1 freeze/thaw cycle have been evaluated.

Specimen Shipping

- Package and label specimens in compliance with applicable local regulations covering the transport of clinical specimens and infectious substances.
- Do not exceed the storage limitations listed above.

Specimen Dilution

- Samples, Renin concentrations above the analytical measuring interval, can be diluted with manual dilution procedure. The recommended dilution ratio is 1:10. The concentration of the diluted sample must be >100 μIU/mL.
- For manual dilution, multiply the result by the dilution factor.
- Please choose applicable diluents or ask Snibe for advice before manual dilution.

PROCEDURE

Materials Provided

Direct Renin (CLIA) assay, control barcode labels.

Materials Required (But Not Provided)

- General laboratory equipment.
- Fully-auto chemiluminescence immunoassay analyzer Maglumi 600, Maglumi 800, Maglumi 1000, Maglumi 2000, Maglumi 2000 Plus, Maglumi 4000, Maglumi 4000 Plus, MAGLUMI X8, MAGLUMI X3, MAGLUMI X6, or Integrated System Biolumi 8000 and Biolumi CX8.
- Additional accessories of test required for the above analyzers include Reaction Module, Starter 1+2, Wash Concentrate, Light Check, Tip, and Reaction Cup. Specific accessories and accessories' specification for each model refer to corresponding Analyzer Operating Instructions.
- Please use accessories specified by Snibe to ensure the reliability of the test results.

Assay Procedure

Preparation of the Reagent

- Take the reagent kit out of the box and visually inspect the integral vials for leaking at the sealing film or elsewhere. If there is no leakage, please tear off the sealing film carefully.
- Open the reagent area door; hold the reagent handle to get the RFID label close to the RFID reader (for about 2s); the buzzer will beep; one beep sound indicates successful sensing.
- Keeping the reagent straight insert to the bottom along the blank reagent track.
- Observe whether the reagent information is displayed successfully in the software interface, otherwise repeat the above two steps.
- 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.

Assay Calibration

- Select the assay to be calibrated and execute calibration operation in reagent area interface. For specific information on ordering calibrations, refer to the calibration section of Analyzer Operating Instructions.
- Execute recalibration according to the calibration interval required in this package insert.

Quality Control

- When new lot used, check or edit the quality control information.
- Scan the control barcode, choose corresponding quality control information and execute testing. For specific information on ordering quality controls, refer to the quality control section of the Analyzer Operating Instructions.

Sample Testing

- After successfully loading the sample, select the sample in interface and edit the assay for the sample to be tested and execute testing. For specific information on ordering patient specimens, refer to the sample ordering section of the Analyzer Operating Instructions.

To ensure proper test performance, strictly adhere to Analyzer Operating Instructions.

Calibration

Traceability: This method has been standardized against WHO Version 4.0 International Standard 68/356.

Test of assay specific calibrators allows the detected relative light unit (RLU) values to adjust the master curve.

Recalibration is recommended as follows:

- Whenever a new lot of Reagent or Starter 1+2 is used.
- Every 14 days.
- The analyzer has been serviced.
- Control values lie outside the specified range.

Quality Control

Controls are recommended for the determination of quality control requirements for this assay and should be run in singlicate to monitor the assay performance. Refer to published guidelines for general quality control recommendations, for example Clinical and Laboratory Standards Institute (CLSI) Guideline C24 or other published guidelines[10].

Quality control is recommended once per day of use, or in accordance with local regulations or accreditation requirements and your laboratory's quality control procedures, quality control could be performed by running the Direct Renin assay:

- Whenever the kit is calibrated.
- Whenever a new lot of Starter 1+2 or Wash Concentrate is used.

Controls are only applicable with MAGLUMI and Biolumi system and only used matching with the same top seven LOT numbers of corresponding reagents. For each target value and range refer to the label.

The performance of other controls should be evaluated for compatibility with this assay before they are used. Appropriate value ranges should be established for all quality control materials used.

Control values must lie within the specified range, whenever one of the controls lies outside the specified range, calibration should be repeated and controls retested. If control values lie repeatedly outside the predefined ranges after successful calibration, patient results must not be reported and 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 package insert.
- If necessary, contact Snibe or our authorized distributors for assistance.

If the controls in kit are not enough for use, please order Direct Renin (CLIA) Controls (REF: 160201428MT) from Snibe or our authorized distributors for more.

RESULTS

Calculation

The analyzer automatically calculates the Direct Renin 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 μIU/mL. For further information please refer to the Analyzer Operating Instructions.

Interpretation of Results

The expected ranges for the Renin assay were obtained by testing 259 upright posture samples and 243 supine posture samples from fasting healthy individuals in China who meet the following inclusion criteria: adult subjects, 18-65 years of age, with normal blood pressure and normal fasting glucose levels. These samples gave the following expected values:

- Upright posture: 4.20-45.6 μIU/mL (5^{th}-95^{th} percentiles).
- Supine posture: 3.11-41.2 μIU/mL (5^{th}-95^{th} percentiles).

Blood was collected between 7:00 a.m. and 10:00 a.m. with the subjects either in an upright or supine position.

Upright samples were collected when individuals sat down to have their blood withdrawn, after standing for 30 minutes; supine samples were collected after the individuals lay in supine position for at least 30 minutes.

Results may differ between laboratories due to variations in population and test method. It is recommended that each laboratory establish its own reference interval.

LIMITATIONS

- The assay is mainly used to aid in the diagnosis and treatment of individuals with suspected or confirmed hypertensive disorders.
- Results should be used in conjunction with patient's medical history, clinical examination and other findings.
- If the Direct Renin results are inconsistent with clinical evidence, additional testing is needed to confirm the result.
- Specimens from patients who have received preparations of mouse monoclonal antibodies for diagnosis or therapy may contain human anti-mouse antibodies (HAMA). Such specimens may show either falsely elevated or depressed values when tested with assay kits which employ mouse monoclonal antibodies [11,12]. Additional information may be required for diagnosis.
- Heterophilic antibodies in human serum can react with reagent immunoglobulins, interfering with *in vitro* immunoassays. Patients routinely exposed to animals or animal serum products can be prone to this interference and anomalous values may be observed [13].
- Bacterial contamination or heat inactivation of the specimens may affect the test results.

SPECIFIC PERFORMANCE CHARACTERISTICS

Representative performance data are provided in this section. Results obtained in individual laboratories may vary.

Precision

Precision was determined using the assay, samples and controls in a protocol (EP05-A3) of the CLSI (Clinical and Laboratory Standards Institute): duplicates at two independent runs per day for 5 days at three different sites using three lots of reagent kits (n = 180). The following results were obtained:

Sample	Mean (μIU/mL) (n=180)	Within-Run		Between-Run		Reproducibility	
		SD (μIU/mL)	%CV	SD (μIU/mL)	%CV	SD (μIU/mL)	%CV
Plasma Pool 1	3.548	0.146	4.11	0.007	0.20	0.186	5.24
Plasma Pool 2	39.370	1.057	2.68	0.968	2.46	1.510	4.50
Plasma Pool 3	349.615	5.799	1.66	4.734	1.35	11.275	3.22
Control 1	40.132	1.288	3.21	0.471	1.17	1.770	4.41
Control 2	107.123	1.985	1.85	0.929	0.87	3.392	3.17

Linear Range

1.00-1000 μIU/mL (defined by the Limit of Quantitation and the maximum of the master curve).

Reportable Interval

0.800-10000 μIU/mL (defined by the Limit of Detection and the maximum of the master curve×Recommended Dilution Ratio).

Analytical Sensitivity

Limit of Blank (LoB) =0.500 μIU/mL.

Limit of Detection (LoD) =0.800 μIU/mL.

Limit of Quantitation (LoQ) =1.00 μIU/mL.

Analytical Specificity

Interference

Interference was determined using the assay, three samples containing different concentrations of analyte were spiked with potential endogenous and exogenous interferents in a protocol (EP7-A2) of the CLSI. The measurement deviation of the interference substance is within ±10%. The following results were obtained:

Interference	No interference up to	Interference	No interference up to
Bilirubin	20 mg/dL	Angiotensin II	1.0 ng/mL
Hemoglobin	500 mg/dL	Propranolol Hydrochloride	230 μg/dL
Intralipid	3000 mg/dL	Spironolactone	60 μg/dL
HAMA	30 ng/mL	Nifedipine	40 μg/dL
ANA	6 (S/CO) strong positive	Lisinopril	32.7 μg/dL
Rheumatoid factor	1500 IU/mL	Losartan potassium	225 μg/dL
Angiotensin I	10 ng/mL		

Cross-Reactivity

Cross-reactivity was determined using the assay, three samples containing different concentrations of analyte were spiked with potential cross-reactants in a protocol (EP7-A2) of the CLSI. The measurement deviation of the interference substance is within ±10%. The following results were obtained:

Cross-reactant	No interference up to	Cross-reactant	No interference up to
Beta2-microglobulin	50 μg/mL	Trypsin	1.6 μg/mL
Cathepsin D	1.5 μg/mL	Plasmin	100 μg/mL

High-Dose Hook

No high-dose hook effect was seen for Direct Renin concentrations up to 150000 μIU/mL.

Method Comparison

A comparison of the Direct Renin assay with a commercially available immunoassay, gave the following correlations (μIU/mL):

Number of samples measured: 117

Passing-Bablok: y=0.9983x-0.1144, τ=0.988

The clinical specimen concentrations were between 2.0 and 984.4 μIU/mL.

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SYMBOLS EXPLANATIONS

	Consult instructions for use		Manufacturer
	Temperature limit (Store at 2-8°C)		Use-by date
	Contains sufficient for<n> tests		Keep away from sunlight
	This way up		Authorized representative in the European Community
	*In vitro* diagnostic medical device		Kit component
	Catalogue number		Batch code
	CE marking with notified body ID number		

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