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Instructions for use

Renin ELISA

REF

MS E-5300



IVD



Introduction

Intended Use

The **Renin ELISA** is an enzyme immunoassay for the quantitative *in vitro diagnostic* measurement of active Renin in human EDTA plasma.

Renin measurements are used in the diagnosis and treatment of certain types of hypertension.

Summary and Explanation

Renin is an enzyme (Mw of 37 kDa) that belongs to the aspartic acid protease family. Renin is a member of Renin-Angiotensin-Aldosterone System (RAAS) that controls blood pressure, renal blood flux, glomerular filtration, and sodium/potassium homeostasis.

Renin is produced constitutively as prorenin, an inactive precursor with 386 amino acids, in the juxtaglomerular cells of the kidney (1). In response to low intra-renal blood pressure, reduced sodium reabsorption, hypokalemia or activity of the sympathetic nervous system, active renin can be released either from a depot in the kidney or generated from prorenin by cleavage of 46 amino acids at the N-terminus of prorenin (2,3). Prorenin secretion into the blood is continuous, in contrast to the tightly controlled release of renin, and blood concentration of prorenin is approx. 100-fold higher than active renin (4,5). After release and activation, soluble renin mediates cleavage of the α_2 -globulin angiotensinogen into the precursor peptide angiotensin I, which ultimately is processed by angiotensin converting enzyme (ACE) to the octapeptide angiotensin II. All actions of angiotensin II are mediated by the G protein-coupled angiotensin type 1 (AT1) and angiotensin type 2 (AT2) receptors (6). Direct physiological effects of Angiotensin II include vasoconstriction, increase of tubular reabsorption of sodium and chloride, water retention, and release of the hormones aldosterone from adrenal cortex, antidiuretic hormone (ADH, Vasopressin) from posterior pituitary, and adrenocorticotrophic hormone (ACTH, Corticotropin) from anterior pituitary. Release of these hormones further supports sodium retention and secretion of potassium/H⁺ in the kidney, and increases thirst sensation and the desire for salt through the subfornical organ of the brain (7,8). In a negative feedback loop, renin secretion is reduced by high concentration of angiotensin II (9), and release of aldosterone is lowered by potassium depletion (10). Beside the action of soluble renin, binding of renin and prorenin to the membrane-bound renin receptor ATP6AP2 in brain, heart, placenta, liver, kidney and pancreas enhances efficiency of angiotensinogen cleavage and induces angiotensin-independent intra-cellular effects by activating mitogen activated kinases ERK1 and ERK2 (11).

Plasma renin is a good index for the activity of the RAAS. In case of dysfunction of the RAAS, the Renin assay will allow clinical implications for diagnosis, treatment, and follow up. Active renin should be measured in:

- Diagnosis of hypertension (high blood pressure: if diastolic blood pressure is > 90 mm Hg and systolic blood pressure is > 140 mm Hg; guideline of the European Society of Cardiology and the European Society of Hypertension)
- Differential diagnosis of hyperaldosteronism (primary hyperaldosteronism, secondary hyperaldosteronism with or without hypertension, pseudo-hyperaldosteronism)
- Diagnosis of isolated deficit in mineral corticoids
- Differential diagnosis of hypokalemia (secondary hyperaldosteronism or primary hypermineralcorticism)
- Detection of Renin producing tumors in the kidney
- Monitoring of glucocorticoid therapy
- Diagnosis of insufficient response to antihypertensive treatment

PRINCIPLE of the test

The Renin ELISA Kit is a solid phase enzyme-linked immunosorbent assay (ELISA) based on the sandwich principle.

The microtiter wells are coated with a monoclonal [mouse] antibody directed towards a unique antigenic site of the human active Renin molecule. An aliquot of patient sample containing endogenous Renin is incubated in the coated well together with Assay Buffer. After incubation, unbound components are washed off. Finally, Enzyme Conjugate, which is a monoclonal anti-Renin antibody conjugated with horseradish peroxidase, is added, and after incubation, unbound enzyme conjugate is washed off.

The amount of bound peroxidase is proportional to the concentration of Renin in the sample.

Having added the substrate solution, the intensity of colour developed is proportional to the concentration of active Renin in the patient sample.

Warnings and Precautions

1. This kit is for in vitro diagnostic use only. For professional use only.
2. All reagents of this test kit which contain human serum or plasma have been tested and confirmed negative for HIV I/II, HBsAg and HCV by FDA approved procedures. All reagents, however, should be treated as potential biohazards in use and for disposal.
3. Before starting the assay, read the instructions completely and carefully. Use the valid version of the package insert provided with the kit. Be sure that everything is understood.
4. The microplate contains snap-off strips. Unused wells must be stored at 2 °C to 8 °C in the sealed foil pouch and used in the frame provided.
5. Pipetting of samples and reagents must be done as quickly as possible and in the same sequence for each step.
6. Use reservoirs only for single reagents. This especially applies to the substrate reservoirs. Using a reservoir for dispensing a substrate solution that had previously been used for the conjugate solution may turn solution colored. Do not pour reagents back into vials as reagent contamination may occur.
7. Mix the contents of the microplate wells thoroughly to ensure good test results. Do not reuse microwells.
8. Do not let wells dry during assay; add reagents immediately after completing the rinsing steps.
9. Allow the reagents to reach room temperature (21-26°C) before starting the test. Temperature will affect the absorbance readings of the assay. However, values for the patient samples will not be affected.
10. Never pipet by mouth and avoid contact of reagents and specimens with skin and mucous membranes.
11. Do not smoke, eat, drink or apply cosmetics in areas where specimens or kit reagents are handled.
12. Wear disposable latex gloves when handling specimens and reagents. Microbial contamination of reagents or specimens may give false results.
13. Handling should be done in accordance with the procedures defined by an appropriate national biohazard safety guideline or regulation.
14. Do not use reagents beyond expiry date as shown on the kit labels.
15. All indicated volumes have to be performed according to the protocol. Optimal test results are only obtained when using calibrated pipettes and microtiter plate readers.
16. Do not mix or use components from kits with different lot numbers. It is advised not to exchange wells of different plates even of the same lot. The kits may have been shipped or stored under different conditions and the binding characteristics of the plates may result slightly different.
17. Avoid contact with *Stop Solution* containing 0.5 M H₂SO₄. It may cause skin irritation and burns.
18. Some reagents contain Proclin 300, BND and/or MIT as preservatives. In case of contact with eyes or skin, flush immediately with water.
19. TMB substrate has an irritant effect on skin and mucosa. In case of possible contact, wash eyes with an abundant volume of water and skin with soap and abundant water. Wash contaminated objects before reusing them. If inhaled, take the person to open air.
20. Chemicals and prepared or used reagents have to be treated as hazardous waste according to the national biohazard safety guideline or regulation.
21. For information on hazardous substances included in the kit please refer to Material Safety Data Sheets. Material Safety Data Sheets for this product are available upon request directly from .

Reagents

Reagents provided

 96

MS E-5331 Microtiterwells

12x8 (break apart) strips, 96 wells; Wells coated with anti-human Renin antibody (monoclonal).

Standards

	Cat. no.	Standard	Concentration	Volume/Vial
STANDARD A	MS E-5301	Standard 0	0 pg/mL	1 ml
STANDARD B	MS E-5302	Standard 1	4 pg/mL	1 ml
STANDARD C	MS E-5303	Standard 2	16 pg/mL	1 ml
STANDARD D	MS E-5304	Standard 3	32 pg/mL	1 ml
STANDARD E	MS E-5305	Standard 4	64 pg/mL	1 ml
STANDARD F	MS E-5306	Standard 5	128 pg/mL	1 ml

The standards are calibrated against WHO 1st International Standard for Renin 68/356
see „Reagent Preparation“

Contain non-mercury preservative.

CONTROL 1

MS E-5351 Control Low

CONTROL 2

MS E-5352 Control High

2 vials, (lyophilized); 1mL; see „Reagent Preparation“

For control values and ranges please refer to vial label or QC-Datasheet.

Contain non-mercury preservative.

ASSAY-BUFF

MS E-5313 Assay Buffer

1 vial, 20 mL, ready to use, Contains non-mercury preservative.

CONJUGATE

MS E-5340 Enzyme Conjugate

1 vial, 14 mL, ready to use, anti-human Renin antibody (monoclonal); HRP conjugated.

Contains non-mercury preservative.

SUBSTRATE

FR E-0055 Substrate Solution

1 vial, 14 mL, ready to use; Tetramethylbenzidine (TMB).

STOP-SOLN

FR E-0080 Stop Solution

1 vial, 14 mL, ready to use; contains 0.5M H₂SO₄.

Avoid contact with the stop solution. It may cause skin irritations and burns.

WASH-CONC 40x

FR E-0030 Wash Solution

1 vial, 30 mL (40X concentrated); see „Preparation of Reagents“.

Note: Additional *Assay Buffer* for sample dilution is available upon request.

Materials required but not provided

- A microtiter plate calibrated reader (450 ± 10 nm)
- Calibrated variable precision micropipettes.
- Absorbent paper.
- Distilled or deionized water
- Timer
- Semi logarithmic graph paper or software for data reduction

Storage Conditions

When stored at 2 °C to 8 °C unopened reagents will retain reactivity until expiration date. Do not use reagents beyond this date.

Opened reagents must be stored at 2 °C to 8 °C. Microtiter wells must be stored at 2 °C to 8 °C. Once the foil bag has been opened, care should be taken to close it tightly again.

Opened kits retain activity for six weeks if stored as described above.

Reagent Preparation

Bring all reagents and required number of strips to room temperature prior to use.

Standards

Reconstitute the lyophilized contents of the standard vial with 1 mL Aqua dest. and let stand for 10 minutes in minimum. Mix the standards several times before use.

Note: The reconstituted standards are stable for 2 days at 2- 8 °C. For longer storage freeze at -20°C.

Controls

Reconstitute the lyophilized content with 1 mL Aqua dest. and let stand for 10 minutes in minimum. Mix the controls several times before use.

Note: The reconstituted controls are stable for 2 days at 2- 8 °C. For longer storage freeze at -20°C.

Wash Solution

Add deionized water to the 40X concentrated Wash Solution.

Dilute 30 mL of concentrated *Wash Solution* with 1170 mL deionized water to a final volume of 1200 mL.

The diluted Wash Solution is stable for 2 weeks at room temperature.

Disposal of the Kit

The disposal of the kit must be made according to the national regulations. Special information for this product is given in the Material Safety Data Sheet.

Damaged Test Kits

In case of any severe damage to the test kit or components, has to be informed in writing, at the latest, one week after receiving the kit. Severely damaged single components should not be used for a test run. They have to be stored until a final solution has been found. After this, they should be disposed according to the official regulations.

SPECIMEN Collection and Preparation

Only EDTA plasma can be used in this assay.

Do not use haemolytic, icteric or lipaemic specimens.

Please note: Samples containing sodium azide should not be used in the assay.

Conditions under which samples are collected must be carefully controlled, since a number of physiological factors can influence the renin secretion. These include:

- Posture: the patient must have been lying down for more than 1 hour or upright for more than 1 hour
- Daily Renin oscillations: sampling is to be done between 7 AM and 10 AM if possible.
- Diet: sodium content in the diet must be known and eventually verified by the measurement of natriuria over a period of 24 hours
- Medication: the level of active renin can be affected by antihypertensive medication (e.g. diuretics, ACE inhibitors, beta adrenergic blocking agents, or vasodilators)
- Pregnancy: the level of inactive and active renin increases during pregnancy
- Menstrual cycle: the level of active renin increases on the second phase of the cycle (sampling is to be done if possible during the first phase)
- Age: active renin level decreases with age

NOTE:

Sera from tumor patients may contain elevated levels of Renin.

Specimen Collection

EDTA plasma

Whole blood should be collected into centrifuge tubes containing anti coagulant and centrifuged immediately after collection.

(E.g. for EDTA plasma Sarstedt Monovette – red cap - # 02.166.001)

Specimen Storage and Preparation

Specimens should be capped and *stored at room temperature and NOT stored at 2-8°C prior to processing*, since cryoactivation of prorenin may occur in the temperature range of 2-8°C, giving false positive active renin values (12,13).

If samples can not be tested within 4 hours of primary collection, store frozen at -20°C or below. It is recommended to rapidly freeze and thaw processed samples avoiding the temperature range of 2-8°C. A dry ice/ethanol bath can be used for rapid freezing procedures.

Specimen Dilution

If in an initial assay, a specimen is found to contain more than the highest standard, the specimens can be diluted with *Assay Buffer* and reassayed as described in Assay Procedure.

For the calculation of the concentrations this dilution factor has to be taken into account.

Example:

- a) dilution 1:2: 75 µL sample + 75 µL *Assay Buffer* (mix thoroughly)
- b) dilution 1:5: 30 µL sample + 120 µL *Assay Buffer* (mix thoroughly).

Assay procedure

General Remarks

- All reagents and specimens must be allowed to come to room temperature before use. All reagents must be mixed without foaming.
- Once the test has been started, all steps should be completed without interruption.
- Use new disposal plastic pipette tips for each standard, control or sample in order to avoid cross contamination.
- Absorbance is a function of the incubation time and temperature. Before starting the assay, it is recommended that all reagents are ready, caps removed, all needed wells secured in holder, etc. This will ensure equal elapsed time for each pipetting step without interruption.
- As a general rule the enzymatic reaction is linearly proportional to time and temperature.

Test Procedure

Each run must include a standard curve.

1.	Secure the desired number of Microtiter wells in the frame holder.
2.	Dispense 150 µL of Assay Buffer in all wells.
3.	Dispense 50 µL of each Standard, Control and samples <u>with new disposable tips</u> into appropriate wells.
4.	Incubate for 90 minutes at room temperature on a plate shaker with ~ 700 rpm.
5.	Briskly shake out the contents of the wells. Rinse the wells 4 times with 300 µL diluted Wash Solution . Strike the wells sharply on absorbent paper to remove residual droplets. Important note: The sensitivity and precision of this assay is markedly influenced by the correct performance of the washing procedure!
6.	Dispense 100 µL Enzyme Conjugate in all wells.
7.	Incubate for 90 minutes at room temperature on a plate shaker with ~ 700 rpm.
8.	Briskly shake out the contents of the wells. Rinse the wells 4 times with 300 µL diluted Wash Solution . Strike the wells sharply on absorbent paper to remove residual droplets.
9.	Add 100 µL of Substrate Solution to each well.
10.	Incubate for 15 minutes at room temperature.
11.	Stop the enzymatic reaction by adding 100 µL of Stop Solution to each well.
12.	Determine the absorbance (OD) of each well at 450 ± 10 nm with a microtiter plate reader. It is recommended that the wells be read within 10 minutes after adding the <i>Stop Solution</i> .

Calculation of Results

1. Calculate the average absorbance values for each set of standards, controls and patient samples.
2. Using semi-logarithmic graph paper, construct a standard curve by plotting the mean absorbance obtained from each standard against its concentration with absorbance value on the vertical (Y) axis and concentration on the horizontal (X) axis.
3. Using the mean absorbance value for each sample determine the corresponding concentration from the standard curve.
4. Automated method: The results in the IFU have been calculated automatically using a 4 PL (4 Parameter Logistics) curve fit. 4 Parameter Logistics is the preferred method. Other data reduction functions may give slightly different results.
5. The concentration of the samples can be read directly from this standard curve. Samples with concentrations higher than that of the highest standard have to be further diluted or reported as > 128 pg/mL. For the calculation of the concentrations this dilution factor has to be taken into account.

Example of Typical Standard Curve

The following data is for demonstration only and **cannot** be used in place of data generations at the time of assay.

Standard	Optical Units (450 nm)
Standard 0 (0 pg/mL)	0.09
Standard 1 (4 pg/mL)	0.19
Standard 2 (16 pg/mL)	0.44
Standard 3 (32 pg/mL)	0.78
Standard 4 (64 pg/mL)	1.14
Standard 5 (128 pg/mL)	2.48

Expected Normal VAlues

It is strongly recommended that each laboratory should determine its own normal and abnormal values.

In a study conducted with apparently normal healthy adults, using the Renin ELISA the following values are observed in plasma:

Population	n	Median (pg/mL)	Mean (pg/mL)	5 – 95 % Range (pg/mL)
Healthy donors upright position	28	7.73	10.97	< 4 – 37.52
Healthy donors supine position	28	6.58	8.30	< 4 – 23.70

The level of active renin in plasma can be affected by antihypertensive medication (e.g. diuretics, ACE inhibitors, beta adrenergic blocking agents, or vasodilators).

The results alone should not be the only reason for any therapeutic consequences. The results should be correlated to other clinical observations and diagnostic tests.

Quality Control

Good laboratory practice requires that controls be run with each calibration curve. A statistically significant number of controls should be assayed to establish mean values and acceptable ranges to assure proper performance.

It is recommended to use control samples according to state and federal regulations. The use of control samples is advised to assure the day to day validity of results. Use controls at both normal and pathological levels. The controls and the corresponding results of the QC-Laboratory are stated in the QC certificate added to the kit. The values and ranges stated on the QC sheet always refer to the current kit lot and should be used for direct comparison of the results.

It is also recommended to make use of national or international Quality Assessment programs in order to ensure the accuracy of the results.

Employ appropriate statistical methods for analysing control values and trends. If the results of the assay do not fit to the established acceptable ranges of control materials patient results should be considered invalid.

In this case, please check the following technical areas: Pipetting and timing devices; photometer, expiration dates of reagents, storage and incubation conditions, aspiration and washing methods.

After checking the above mentioned items without finding any error contact your distributor or the manufacturer directly.

Performance Characteristics

Assay Dynamic Range

The range of the assay is between 0.81 – 128 pg/mL.

Specificity of Antibodies (Cross-Reactivity)

The following substances were tested for cross-reactivity of the assay:

Mean cross reactivity with Prorenin was 0.69 % (mean value when prorenin was spiked in a concentration range from 256 – 4096 pg/mL). However, the observed cross reactivity may only represent a contamination of the recombinant prorenin preparation with active renin due to auto-activation.

Cross-reactivity was not detectable against human serum albumin, human gamma globulin, human hepcidine, and pepsin.

Sensitivity

The analytical sensitivity of the ELISA was calculated by adding 2 standard deviations to the mean of 20 replicate analyses of the Zero Standard (S0) and was found to be 0.81 pg/mL.

Reproducibility

Intra Assay

The within assay variability is shown below:

Sample	1	2	3
Mean (pg/mL)	9.12	26.98	43.99
CV (%)	8.73	3.88	4.24
n =	20	20	20

Inter Assay

The between assay variability is shown below:

Sample	1	2	3
Mean (pg/mL)	19.28	36.20	66.72
CV (%)	8.88	6.27	5.19
n =	12	12	12

Recovery

Samples have been spiked by adding Renin solutions with known concentrations in a 1:1 ratio.

The % recovery has been calculated by multiplication of the ratio of the measurements and the expected values with 100 (expected value = (endogenous Renin + added Renin) / 2; because of a 1:2 dilution of plasma with spike material).

		Sample 1	Sample 2	Sample 3
Concentration [pg/mL]		16.71	40.21	15.97
Average Recovery		92.92	95.09	96.00
Range of Recovery [%]	from	85.99	87.93	86.83
	to	105.47	101.37	105.25

Linearity

	Sample 1	Sample 2	Sample 3
Concentration [pg/mL]	45.16	53.20	126.0
Average Recovery	101.7	102.8	98.5
Range of Recovery [%]	from	96.7	95.6
	to	108.6	114.6

Limitations of Use

Reliable and reproducible results will be obtained when the assay procedure is performed with a complete understanding of the package insert instruction and with adherence to good laboratory practice.

Any improper handling of samples or modification of this test might influence the results.

Interfering Substances

Haemoglobin (up to 1 mg/mL), Bilirubin (up to 0.5 mg/mL) and Triglyceride (up to 30 mg/mL) have no influence on the assay results. Cross-reactivity

Drug Interferences

Until today no substances (drugs) are known to us, which have an influence to the measurement of Renin in a sample. However, the level of active renin in plasma can be affected by antihypertensive medication (e.g. diuretics, ACE inhibitors, beta adrenergic blocking agents, or vasodilators)

High-Dose-Hook Effect

No hook effect was observed in this test up to 8,200 pg/mL of Renin.

Legal Aspects

Reliability of Results

The test must be performed exactly as per the manufacturer's instructions for use. Moreover the user must strictly adhere to the rules of GLP (Good Laboratory Practice) or other applicable national standards and/or laws. This is especially relevant for the use of control reagents. It is important to always include, within the test procedure, a sufficient number of controls for validating the accuracy and precision of the test.

The test results are valid only if all controls are within the specified ranges and if all other test parameters are also within the given assay specifications. In case of any doubt or concern please contact .

Therapeutic Consequences

Therapeutic consequences should never be based on laboratory results alone even if all test results are in agreement with the items as stated under point 11.1. Any laboratory result is only a part of the total clinical picture of a patient.

Only in cases where the laboratory results are in acceptable agreement with the overall clinical picture of the patient should therapeutic consequences be derived.

The test result itself should never be the sole determinant for deriving any therapeutic consequences.

Liability

Any modification of the test kit and/or exchange or mixture of any components of different lots from one test kit to another could negatively affect the intended results and validity of the overall test. Such modification and/or exchanges invalidate any claim for replacement.

Claims submitted due to customer misinterpretation of laboratory results subject to point 11.2. are also invalid. Regardless, in the event of any claim, the manufacturer's liability is not to exceed the value of the test kit. Any damage caused to the test kit during transportation is not subject to the liability of the manufacturer.

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Symbols:

	Storage temperature		Manufacturer		Contains sufficient for <n> tests
	Expiry date		Batch code		For in-vitro diagnostic use only!
	Consult instructions for use		Content		CE labelled
	Caution		Catalogue number		For research use only!