EIASON® Renin

IVD

Enzymeimmunoassay for the quantitative in vitro diagnostic measurement of active Renin in human serum and plasma

Kit instruction

For in-vitro use only

Product of

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REF **E03-005-96Q**

2 °C 8 °C

CE

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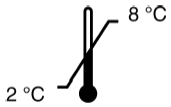
Used IFU-Symbols

Symbol	English	Symbol	English
IVD	In vitro diagnostic device	SUB	Substrate
REF	Order number	CAL 0-5	Calibrators
	Product of	STOP	Stop solution
	Storage	WASH	Wash buffer
CE	European Conformity	LOT	Batch code
	Expiry date	MPL	Microplate
CONJ	Conjugate	CO1 CO2	Control Low Control High
BU	Assay buffer		

Intended use

For in-vitro use only.

The EIASON® Renin ELISA is an enzyme immunoassay for the quantitative in vitro diagnostic measurement of active Renin in human serum and plasma.
Renin measurements are used in the diagnosis and treatment of certain types of hypertension.

Summary

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 a_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 (AT_2) 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 adrenocorticotropic 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

Assay principle

The EIASON® 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 BU. 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

The EIASON® Renin ELISA Kit is for in vitro diagnostic use only and is not for internal use in humans or animals. This product must be used strictly in accordance with the instructions set out in the Package Insert. IASON will not be held responsible for any loss or damage (except as required by statute) caused, arising out of non-compliance with the instructions provided.
CAUTION: this kit contains material of human and/or animal origin. Handle kit reagents as if capable of transmitting an infectious agent.
Source material from Human origin which is used in this kit was tested and found negative for HbsAG and HIV as well as for HCV antibodies. However, since there is no diagnostic procedure that excludes these agents with 100 percent certainty all components should be handled as potentially hazardous material.
Appropriate precautions and good laboratory practices must be used in the storage, handling and disposal of the kit reagents. Disposal of kit reagents should be in accordance with local regulations.

Storage and stability

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.

Specimen collection and storage

Serum or plasma (EDTA- or heparin 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, vasodilators, renin inhibitors)
− 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

Serum:
Collect blood by venipuncture , allow to clot, and separate serum by centrifugation at room temperature. Do not centrifuge before complete clotting has occurred. Patients receiving anticoagulant therapy may require increased clotting time.

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

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 cannot 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 BU 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 BU (mix thoroughly)
b) dilution 1:5: 30 µL sample + 120 µL BU (mix thoroughly).

Materials provided

Allow all reagents 1-11 to reach room temperature before use.

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

2. CAL0-5 Calibrators, 6 vials, (lyophilized); to be reconstituted with 1 mL distilled water end let stand for at least 10 min. Mix the standards several times before use.
   Note: The reconstituted standards are stable for 14 days at 2 °C - 8 °C. For longer storage freeze at -20 °C.
   Concentrations: 0; 4; 16; 32; 64; 128 pg/mL
   Conversion: 1 pg/mL = 1.44 µIU/mL
   The standards are calibrated against WHO 1st International Standard for Renin 68/356.

3. CO1 CO2 2 vials, (lyophilized); to be reconstituted with 1 mL distilled water end let stand for at least 10 min. Mix the standards several times before use.
4. *Note: The reconstituted standards are stable for 14 days at 2 °C - 8 °C. For longer storage freeze at -20 °C.*
   For control values and ranges please refer to vial label or QC-Datasheet.

5. BU Assay Buffer, 1 vial, 20 mL, ready to use,

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

7. SUB Substrate Solution, 1 vial, 14 mL, ready to use,
   Tetramethylbenzidine (TMB).

8. STOP Stop Solution, 1 vial, 14 mL, ready to use,contains 0.5M H_2SO_4, avoid contact with the stop solution. It may cause skin irritations and burns.

9. WASH Wash Solution, 1 vial, 30 mL (40x concentrated),
   Dilute 30 mL of concentrated WASH with 1170 mL deionized water to a final volume of 1200 mL. *The diluted WASH is stable for 2 weeks at room temperature*.

Note: Additional BU for sample dilution is available upon request.

Materials required but not provided

- A microtiter plate calibrated reader (450 ± 10 nm) (e.g. the IASON Instruments Microtiter Plate Reader).
- Calibrated variable precision micropipettes.
- Absorbent paper.
- Distilled or deionized water
- Timer
- Semi logarithmic graph paper or software for data reduction

Assay procedure

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.

Each run must include controls and a standard curve.

1. Secure the desired number of MPL in the frame holder.
2. Dispense 150 µL of BU in all wells.
3. Dispense 50 µL of each CAL0-5, CO1-2 and samples with new disposable tips into appropriate wells.
4. Incubate for 90 minutes at room temperature on a plate shaker with 300 - 700 rpm.
5. Briskly shake out the contents of the wells.
   Rinse the wells 4 times with 300 µL diluted WASH. 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 CONJ in all wells.
7. Incubate for 90 minutes at room temperature on a plate shaker with 300 - 700 rpm.
8. Wash 3 times with diluted WASH as described under point 5.
9. Add 100 µL of SUB to each well.
10. Incubate for 15 minutes at room temperature.
11. Stop the enzymatic reaction by adding 100 µL of STOP 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 STOP.

or fully automated on:

- **IASON® Quardette**
- **IASON® PersonalLab**
- **IASON® Gladiator**

Calculation of results

- Draw a standard curve by plotting the absorbance of each standard against its concentration. Read off the values of the test samples. Samples with concentrations higher than that of the highest standard have to be further diluted. For the calculation of the concentrations this dilution factor has to be taken into account.
- Alternative data reduction techniques may be employed but users should confirm that the selected curve fit is appropriate and gives acceptable results. 4PL (4 parameter logistics) is recommended.

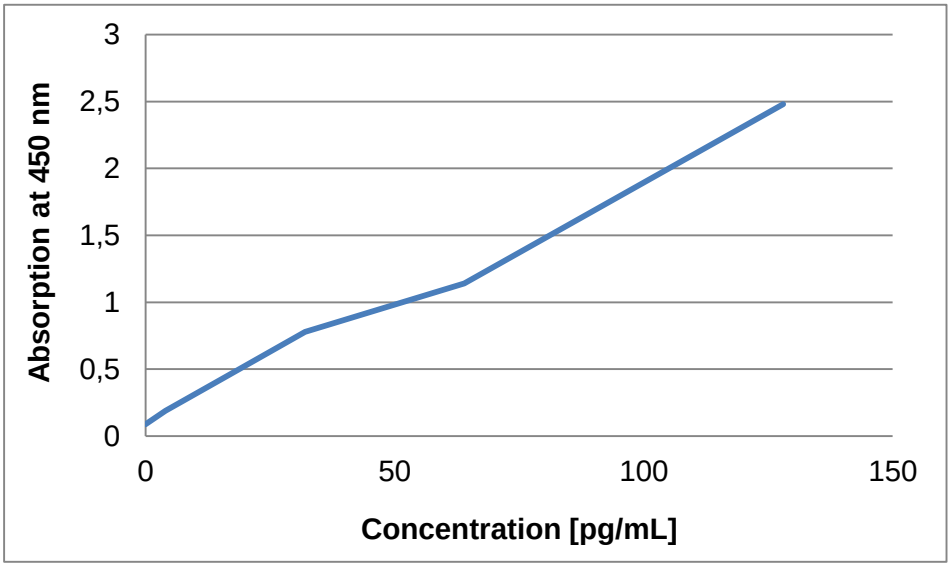
Sample assay data

Typical results obtained with EIASON® Renin calibrators:

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

This data is for illustration only and must not be used for the calculation of any sample result.

Typical calibration curve



This sample calibration curve is for illustration only.

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 IASON GmbH ELISA the following values are observed in plasma:

Expected Values

Population	n	Mean [pg/mL]	Median [pg/mL]	99th percentile [pg/mL]	95th percentile [pg/mL]	5th percentile [pg/mL]	1st percentile [pg/mL]
Healthy donors suspine position	26	17.72	15.31	35.64	31.90	4.66	2.99
Healthy donors upright position	26	23.95	23.27	47.85	42.30	7.54	3.84

In a study conducted with apparently normal healthy adults, using the EIASON® Aldosterone ELISA and the EIASON® Renin ELISA the following *Aldosterone-Renin Ratios* were determined in plasma:

Ratio aldosterone/renin (pg/mL / pg/mL)

n	Mean	Median	99th percentile	95th percentile	5th percentile	1st percentile
89	8.68	5.30	49.65	28.06	0.68	0.45

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 IASON GmbH 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 EIASON® Renin ELISA was calculated by adding 2 standard deviations to the mean of 20 replicate analyses of the CAL0 and was found to be 0.81 pg/mL.

Precision

Intra-assay-precision (n = 20)			Inter-assay-precision (n = 20)		
Sample	**Mean [pg/mL]**	**CV [%]**	**Sample**	**Mean [pg/mL]**	**CV [%]**
1	9.12	8.73	1	19.28	8.88
2	26.98	3.88	2	36.20	6.27
3	43.99	4.24	3	66.72	5.19

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	94.9
	to	108.6	114.6	100.8

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.

Drug interferences

The renin inhibitor aliskiren will increase active renin immunoreactivity in a dose-dependant manner, from 0.54 µM (+ 121%) up to 540 µM (+151%).
In addition, 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 IASON GmbH.

Therapeutic consequences

Therapeutic consequences should never be based on laboratory results alone. 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 *Therapeutic consequences* 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.

REFERENCES / Literature

1. Imai T, Miyazaki H, Hirose S, et al. Cloning and sequence analysis of cDNA for human renin precursor. Proc. Natl. Acad. Sci. (1983) 80, 7405–7409.
2. Reudelhuber TL, RamLa D, Chiu L, et al. Proteolytic processing of human prorenin in renal and non-renal tissues. Kidney Int. (1994) 46, 1522–1524.
3. Neves FA, Duncan KG, Baxter JD. Cathepsin B is a prorenin processingenzyme. Hypertension (1996) 27, 514 –517.
4. Müller DN, Luft FC. Direct renin inhibition with aliskiren in hypertension and target organ damage.
   Clin J Am Soc Nephrol. (2006) 1, 221-8.
5. Toffelmire EB, Slater K, Corvol P, et al. Response of plasma prorenin and active renin to chronic and acute alterations of renin secretion in normal humans. Studies using a direct immunoradiometric assay. J Clin Invest. (1989) 83, 679–687.
6. Carey RM, Padia SH. Angiotensin AT2 receptors: control of renal sodium excretion and blood pressure. Trends Endocrinol Metab. (2008) 19, 84-7.
7. Koeppen BM, Stanton BA. Renal Physiology (4th ed.). Philadelphia, PA. Mosby Physiology Monograph Series, 2007.
8. Navar LG, Inscho EW, Majid DSA, et al. Paracrine regulation of the renal microcirculation.
   Physiol. Rev. (1996) 76, 425–536.
9. Müller MW, Todorov V, Krämer BK, Kurtz A. Angiotensin II inhibits renin gene transcription via the protein kinase C pathway. Pflugers Arch. (2002) 444, 499-505.
10. Spät A, Hunyady L. Control of aldosterone secretion: a model for convergence in cellular signaling pathways. Physiol Rev. (2004) 84, 489-539.
11. Nguyen G., Delarue F., Burcklé C., et al. Pivotal role of the renin/prorenin receptor in angiotensin II production and cellular responses to renin. J Clin Invest. (2002) 109, 1417–1427.
12. Pitarresi TM., Rubattu S, Heinrikson R, Sealey JE. Reversible cryoactivation of recombinant human prorenin. J.Biol.Chem. (1992) 267, 11753-9.
13. Nicar MJ. Specimen processing and renin activity in plasma. Clin. Chem. (1992) 38, 598.

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Pipetting scheme

Allow all reagents to reach room temperature before use.

1. Pipetting	BU			**150µL**
2. Pipetting	CAL 0-5	CO1	CO2	Samples
	50 µL	**50 µL**		**50µL**
3. Incubation	90 min at room temperature on a shaker with 300 - 700 rpm			
4. Washing	wash 4 x : aspirate or decant add 300 µL diluted WASH aspirate or decant and dry on an absorbent material			
5. Pipetting	CONJ			**100µL**
6. Incubation	90 min at room temperature on a shaker with 300 - 700 rpm			
7. Washing	wash 4 x : aspirate or decant add 300 µL diluted WASH aspirate or decant and dry on an absorbent material			
8. Pipetting	SUB			**100µL**
9. Incubation	15 min at room temperature			
10. Pipetting	STOP			**100µL**
11. Reading	450nm (RF 620 – 650nm) Optional Overrange Filter: 405nm, Factor: 3 (dependent on photometer), reading within 10 min. Calculation: 4-parameter or point to point			

Expected Values

Population	n	Mean [pg/mL]	Median [pg/mL]	99th percentile [pg/mL]	95th percentile [pg/mL]	5th percentile [pg/mL]	1st percentile [pg/mL]
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