Human Renin (High sensitive) ELISA Kit

Enzyme Immunoassay for the quantitative determination of human Renin in serum and plasma

Catalog number: ARG80884

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INTRODUCTION

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. 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. 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. After release and activation, soluble renin mediates cleavage of the a2-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. 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 thirstsensation and the desire for salt through the subfornical organ of the brain. In a negative feedback loop, renin secretion is reduced by high concentration of angiotensin II, and release of aldosterone is lowered by potassium depletion. 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.

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 ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. An antibody specific for Renin has been pre-coated onto a microtiter plate. Standards or samples are pipetted into the wells and any Renin present is bound by the immobilized antibody. After washing away any unbound substances, an HRP-Renin antibody conjugate is added to each well and incubate. After washing away any unbound antibody-enzyme reagent, a substrate solution (TMB) is added to the wells and color develops in proportion to the amount of Renin bound in the initial step. The color development is stopped by the addition of acid and the intensity of the color is measured at a wavelength of 450 nm ±2 nm. The concentration of Renin in the sample is then determined by comparing the O.D of samples to the standard curve.

MATERIALS PROVIDED & STORAGE INFORMATION

Store the unopened kit at 2-8 °C. Use the kit before expiration date.

Component	Quantity	Storage information
Antibody-coated microplate	12 strips x 8-well	4°C
HRP-antibody Conjugate	14 ml, ready to use	4°C
Standards 0-5 (0, 4, 16, 32, 64, 128 pg/ml)	6 vials, lyophilized	4°C
Control Low (11.85 pg/ml, acc. range: 7.7-16.00 pg/ml)	1 vial, lyophilized	4°C
Control High (71.18 pg/ml, acc. range: 46.27-96.09 pg/ml)	1 vial, lyophilized	4°C
Assay buffer	20 ml, ready to use	4°C
40X Wash buffer	30 ml	4°C
Substrate (TMB)	14 ml, ready to use	4°C
Stop solution	14 ml, ready to use	4°C

MATERIALS REQUIRED BUT NOT PROVIDED

- Microplate reader capable of measuring absorbance at 450nm
- Microplate shaker (shaking amplitude 3 mm; approx. 300-700 rpm), or an orbital shaker. If other kind of shaker is used, the rotating speed may need to be optimized by user.
- Pipettes and pipette tips
- Deionized or distilled water
- Automated microplate washer (optional)

TECHNICAL HINTS AND PRECAUTIONS

- Wear protective gloves, clothing, eye, and face protection especially while handling blood or body fluid samples.
- Store the kit at 4°C at all times.
- All kit reagents and specimens should be brought to room temperature and mixed gently but thoroughly before use. Avoid repeated freezing and thawing of reagents and specimens.
- If crystals are observed in the 40X Wash buffer, warm to RT or 37°C until the crystals are completely dissolved.
- Once the test has been started, all steps should be completed without interruption. Make sure that the required reagents, materials and devices are prepared ready at the appropriate time.
- Ensure complete reconstitution and dilution of reagents prior to use.
- The controls should be included in each run and fall within established confidence limits. The confidence limits are listed in the QC-Report.
- Do not let wells dry during assay; add reagents immediately after

completing the rinsing steps.

- It is highly recommended that the standards, samples and controls be assayed in duplicates.
- Change pipette tips between the addition of different reagent or samples.
- Samples contain azide cannot be assayed.

SAMPLE COLLECTION & STORAGE INFORMATION

The sample collection and storage conditions listed below are intended as general guidelines. Sample stability has not been evaluated.

Serum - Use a serum separator tube (SST) and allow samples to clot for 30 minutes before centrifugation for 15 minutes at 1000 x g. Remove serum and assay immediately within 4 hours of primary collection or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

Plasma - Collect plasma using EDTA as an anticoagulant. Centrifuge for 15 minutes at 1000 x g within 30 minutes of collection. Assay immediately within 4 hours of primary collection or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

- Storage: 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. 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.

- Note for sample collection:

a. Samples containing sodium azide should not be used in the assay. Do not use haemolytic, icteric or lipaemic specimens.

b. 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

REAGENT PREPARATION

- 1X Wash buffer: Dilute 40X wash buffer with distilled water to yield 1X wash buffer. The diluted Wash Solution is stable for 2 weeks at room temperature.
- Controls: Reconstitute the lyophilized content with 1.0 mL distilled water and let stand for 10 minutes in minimum. Mix the controls several times before use. The reconstituted controls are stable for 14 days at 2-8 °C. For longer storage freeze at-20 °C.
- Standards: Reconstitute the lyophilized contents of the standard vial with 1.0 mL distilled water and let stand for 10 minutes in minimum. Mix the standards several times before use. The reconstituted standards are stable for 14 days at 2-8°C. For longer storage freeze at-20°C.
- Sample: 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

All materials should be equilibrated to room temperature (RT) before use. Standards, samples and controls should be assayed in duplicates.

1. Remove excess microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, and reseal it.
2. Add 150 µl Assay Buffer into all wells.
3. Add 50 µl standards, controls and samples into each well.
4. Incubate for 90 minutes at RT on a microplate shaker with 300-700 rpm.
5. Aspirate each well and wash, repeating the process 3 times for a total 4 washes. Wash by filling each well with wash buffer (300 µl) using a squirt bottle, manifold dispenser, or autowasher. Complete removal of liquid at each is essential to good performance. After the last wash, remove any remaining distilled water by aspirating, decanting or blotting against clean paper towels.

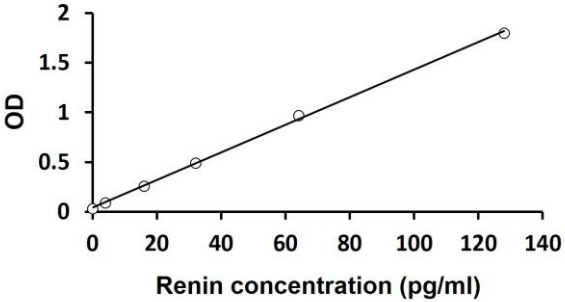
   Note: The sensitivity and precision of this assay is markedly influenced by the correct performance of the washing procedure!
6. Add 100 µl HRP-antibody conjugate into each well.
7. Incubate for 90 minutes at RT on a microplate shaker with 300-700 rpm.
8. Aspirate and wash wells as step 5.
9. Add 100 µl of TMB Substrate to each well. Incubate for 15 minutes at room temperature in dark.
10. Add 100 µl Stop solution into each well.
11. Read the OD with a microplate reader at 450 nm immediately. It is recommended that the wells be read within 10 minutes after adding the Stop Solution.

CALCULATION OF RESULTS

1. Calculate the average absorbance values for each set of standards, controls and patient samples.
2. Using linear 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. The diluted samples must be further converted by the appropriate dilution factor according to the sample preparation procedure as described above.
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.
6. Conversion: 1 pg/mL = 1.44 µIU/mL.

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.



QUALITY ASSURANCE

Sensitivity

The minimal detectable concentration is approximately 0.81 pg/ml.

Specificity

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

Mean cross reactivity with Prorenin was 0.71% (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.

Intra-assay and inter-assay precision

The CV value of intra-assay precision was 5.62% and the CV value of inter-assay precision was 6.78%.

Recovery

85.99-105.47%

Linearity

94.9-114.6%

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 may be affected by antihypertensive medication (e.g. diuretics, ACE inhibitors, beta adrenergic blocking agents, or vasodilators)