



1. NAME AND INTENDED USE

RENIN III GENERATION is a radioimmunoassay kit for the quantitative determination of active renin in human plasma.

2. INTRODUCTION

Renin is a proteolytic acidic enzyme produced and secreted by the juxtaglomerular cells. It cleaves angiotensinogen into angiotensin I (inactive), which ultimately leads to the production of angiotensin II (active).

Therefore, renin, which has a limiting effect on the production of angiotensin, is a key-factor in the regulation of arterial pressure and hydrosodic metabolism.

As most enzymes which act outside of the cells in which they are synthesized, renin exists in both inactive and active forms. Inactive renin (prorenin) which is found in plasma, amniotic fluid and in the kidney, can be activated in different ways (cryoactivation, acidification or partial proteolysis) which expose the active site of the enzyme. Inactive renin can account for up to 90 % of the total renin in the circulation.

However, it is the active renin which provides the medium through which biological activity takes place.

Now, human active renin is well known : it is a polypeptidic chain of 345 amino acids, with a molecular weight of about 40,000.

Angiotensinogen, the substrate of renin, is a liver protein from which angiotensin I is produced.

It must be noted that the concentration of angiotensinogen in circulation influences the level of plasmatic renin activity and so, ultimately, the level of angiotensin II. This shows the importance, for the synthesis of angiotensinogen, of those factors directly active at the liver level.

It has been proved that the in vivo hypertensive action of angiotensin I is due to its conversion into angiotensin II by a carboxypeptidase (converting enzyme). The converting enzyme is regulated by the glucocorticoids and the thyroid hormones. Angiotensin II is the major effector in the renin-angiotensin system, maintaining circulatory homeostasis through its direct effect on the smooth vascular muscle and on the stimulation of aldosterone, and by its stimulatory effect on the sympathetic nervous system.

In the kidney, angiotensin II is involved in the control of glomerular filtration and in renal blood flux. Renin is secreted by the kidneys in response to a reduction in the perfusion of the renal artery (intrarenal baroreceptor), a reduction of distal tubular reabsorption of Na^+ (sodium leakage), an hypokaliemia or a B-adrenergic stimulation. In addition renin secretion is reduced (negative feedback) when there is a high plasmatic concentration of angiotensin II.

RENIN ASSAY IS NECESSARY IN HYPERTENSIVE PATIENTS AND IN THE THERAPEUTICAL FOLLOW UP OF HIGH BLOOD PRESSURE.

Renin should be measured :

- Whenever diastolic blood exceeds 110 mm Hg (to trace an hypertension of renal origin).
- Whenever there is an hypokaliemia ($< 3.8 \text{ mmol/l}$) : to try to find a secondary hyperaldosteronism or a primary hypermineralocorticoidism.
- Whenever the response to antihypertensive treatment is insufficient.
- In order to determine the functional character of a renal artery stenosis (by measurement of renin in the renal veins during acute inhibition of the converting enzyme).
- Whenever a cancer is linked to an increase in blood pressure (to look for ectopic production of renin).

3. PRINCIPLE

RENIN III Generation is an immunoradiometric (sandwich technique) with following characteristics :

- Its uses a pair of antirenin Monoclonal Antibodies (MAb), selected on the basis of three very precise criteria : specificity, avidity and complementarity.
- The first monoclonal antibody, coated on polystyrene tubes, specifically recognizes both the active and inactive forms of renin.
- The second monoclonal antibody labelled with 125 iodine, specifically recognizes the active form of renin.

The assay involves the following steps :

1. Incubation of standard and unknown sera in the presence of an excess of the first insolubilized monoclonal antibody on the wall of polystyrene tubes and of an excess of the second iodine 125 monoclonal antibody.
2. Washing for elimination of the free fraction and measurement of activity bound to the solid phase.

**4. REAGENTS**

Each kit contains enough reagents for 100 tubes. The expiry date is marked on the external label.

REAGENTS	QUANTITY	STORAGE
COATED TUBES (R1) : ready for use. Mouse monoclonal antibody anti human renin coated on the bottom of the tube.	2 packs of 50 tubes	2-8°C until the expiry date. After packaging opening, unused antibodies coated tubes must be stored in the plastic bag.
ANTI-RENINE ¹²⁵I (R2) : ready for use. Mouse anti-human active renin monoclonal antibody ¹²⁵ I in a Tris buffer pH 7.9 containing horse serum and 0,1 % sodium azide (NaN ₃). One vial contains around 360 kBq with a specific radioactivity of 925 kBq/μg so that 210 000 dpm can be obtained for 100 μl at the day of the labelling.	1 11 ml vial	2-8°C until the expiry date.
STANDARD (S0) : ready for use. Phosphate buffer pH 7.4 containing casein, 0,1 % sodium azide and a dye.	1 7 ml vial	2-8°C until the expiry date.
STANDARDS (S1 – S5) : lyophilized. (*) Active renin diluted in S0, containing 0,1 % sodium azide. 2.5 – 5 – 20 - 80 - 320 pg/ml. Reconstitute with 3 ml of demineralised or distilled water. After complete dissolution it must be carefully homogenized. Avoid foam formation during homogenization.	5 qs 3 ml vials	2-8°C until the expiry date. After reconstitution, standards can be used within 4 hours at room temperature (18-25°C). Then, they can be kept 10 days at 2-8°C or aliquoted and kept frozen at -20°C for 6weeks. (***)
CONTROL (C) : lyophilized. (**) Sample of human origin containing 0.1 % sodium azide. Reconstitute with 2 ml of demineralised or distilled water. After complete dissolution it must be carefully homogenized. Avoid foam formation during homogenization.	1 qs 2 ml vial	2-8°C until the expiry date. After reconstitution, control sample must be aliquoted and rapidly kept frozen at -20°C. In this condition it is stable 6 weeks. (***)
WASHING SOLUTION (R3) : Imidazole-buffer pH 7.4 with Tween 20 and sodium azide (0,1 %). Dilute the content in 975 ml of demineralised or distilled water. Mix before use.	1 25 ml vial	2-8°C until the expiry date. After reconstitution : 6 weeks at 2-8°C.
PLASTIC BAG	1	

(*) The values shown above are only target values : the true value of each standard or control is shown on its label.
Calibration on MRC RENIN : 1 pg = 1.8.10⁶ IU, WHO 68/356.

(**) The acceptance range true values are printed on the vial label.

(***) Standards and control could be frozen and thawed once.

5. PRECAUTIONS FOR USE**5.1. Safety measures**

Raw materials of human origin contained in the reagents of this kit have been tested with licensed kits and found negative for the anti-HIV 1, anti-HIV 2, anti-HCV antibodies and the HBs antigen. However as it is impossible to strictly guarantee that such products will not transmit hepatitis, the HIV virus, or any other viral infection, all raw materials of human origin including the samples to be assayed must be treated as potentially infectious.

Do not pipette by mouth.

Do not smoke, eat or drink in areas in which specimens or kit reagents are handled.

Wear disposable gloves while handling kit reagents or specimens and wash hands thoroughly afterwards.

Avoid splashing.

Decontaminate and dispose of specimens and all potentially contaminated materials as if they contained infectious agents. The recommended method of doing this is autoclaving for a minimum of one hour at 121.5°C.

Sodium azide may react with lead or copper piping to form highly explosive metal azides. During waste disposal, flush the drains thoroughly to prevent a build-up of these products.

5.2. Basic radioprotection rules

This radioactive product may only be received, purchased, stored or used by persons so authorized, and by laboratories covered by such authorization. The solution should under no circumstances be administered to humans or to animals.

The purchase, storage, use or exchange of radioactive products are subject to the laws in force in the user's country.

The enforcement of the basic rules for handling radioactive products ensures adequate security.

A summary of these is given below :

Radioactive products must be stored in their original containers in a suitable area.

A record of the reception and storage of radioactive products must be kept up to date.

Handling of radioactive products should take place in a suitably-equipped area with restricted access (controlled zone).

Do not eat, drink, smoke or apply cosmetics in a controlled zone.

Do not mouth-pipette radioactive solutions.

Avoid any direct contact with all radioactive products by using laboratory coats and protective gloves.



Contaminated laboratory equipment and glassware must be disposed of immediately after contamination to prevent cross-contamination of different isotopes.

Any contamination or radioactive substance loss should be dealt with in accordance with the established procedures.

All radioactive waste disposal must be carried out according to the regulations in force.

5.3. Handling precautions

Do not use kit components beyond their expiry date.

Do not mix reagents from different batches.

Avoid any microbial contamination of the reagents or of the water.

Fully respect the incubation conditions and the washing instructions indicated.

6. SPECIMEN COLLECTION AND PREPARATION

The assay is performed directly on EDTA plasma.

If the test is not run within 4 hours following sampling, samples must be aliquoted and stored deep frozen at - 20°C. Under these conditions samples can be stored 4 weeks. After thawing plasma must be shaken and carefully centrifuged to eliminate any trace of fibrin.

Warning :

Do not use highly hemolyzed or severely lipemic samples.

Avoid successive freezing and thawing.

Do not store plasma at + 4°C as possible activation may occur.

Dilutions

Should elevated renin (> 320 pg/ml) levels be suspected, the standard 0 found in the kit is used for dilution.

It is recommended that disposable plastic tubes be used when carrying out dilutions.

7. LIMITATION OF THE TEST PROCEDURE

The significance of active renin measurements can be meaningfully interpreted only when the patient is studied under controlled conditions and with defined sodium balance.

Since a number of physiological factors can influence the renin secretion, conditions under which samples are collected must be carefully controlled :

- the patient must not have taken any antihypertensive medication for 8 days.
- his posture must be controlled : he must have been lying down for more than one hour or upright for more than one hour.
- sodium content in the diet must be known and eventually verified by the measurement of natriuria over a period of 24 hours (60 to 200 mEq/24 h).
- It must be known that physiological factors affect renin secretion :
- both levels of inactive and active renin increase during pregnancy,
 - * menstrual cycle : increase of the level on the second phase of the cycle (sampling is to be done if possible during the first phase),
 - * active renin level decreased with age,
 - * Nycthemeral cycle affects also the concentration : sampling is to be done between 7 AM and 10 AM if possible.

It must be also noted that various medications could affect the renin secretion :

Diuretics, inhibitors of the conversion enzyme (Captopril, Enalapril,...), vasodilators (Dihydralazine, Minoxidil, Prazosine,...) could provide a stimulation of the renin-angiotensin system.

Beta adrenergic-blocking agents (Labetalol,...), Clonidin, Methyl-dopa,... could provide inhibition on the renin-angiotensin system.

8. TEST PROCEDURE

8.1. Material required

Precision micropipettes or similar with disposable tips, capable of dispensing 100 µl, 300 µl and 3 ml (± 1 %). Their calibration should be checked regularly.

Distilled or demineralized water. Disposable plastic tubes. Graduated cylinder (1 liter). Circular horizontal shaker. Appropriate racks. Parafilm®. Gamma scintillation counter calibrated for 125 iodine measurement. Equipment suitable for this assay is available from CIS bio international ; information on request.

8.2 Protocol

All reagents must be brought to room temperature (18-25°C) at least 30 minutes before their use.

Dispensing of the reagents into the tubes is also carried out at room temperature.

The assay requires the following groups of tubes : Tube T for the Total Activity, standard "0" group for the determination of non-specific binding, Standard groups to establish the standard curve, Control group for the control, Sx groups for the samples to be assayed.

It is recommended to perform the assay in duplicate for standards, control and samples.

If necessary carefully centrifuged thawed samples.

A standard curve should be performed on each test occasion.

Observe the order in which reagents have to be added :

Dispense 300 µl of standards, control or samples into the corresponding groups of tubes.

Add 100 µl of tracer ¹²⁵I into all tubes. Close tubes T.

Mix gently. Cover with Parafilm®.

Incubate for 3 hours at room temperature (18-25°C) under constant horizontal agitation.



Wash the coated tubes as follows :

Discard the reactive medium by aspiration (or by inversion), except tubes T.

Add 2.0 ml of washing solution (R3 diluted to 1/40 in distilled or demineralised water) to each tube, except tubes T.

Discard the washing solution by aspiration (or by inversion).

Repeat the process twice.

Then, leave the tubes to stand 2 minutes on the inverting position or aspirate the contents of the tubes as completely as possible. There must be no residual volume in the coated tubes after washing.

To obtain reliable and reproducible results, the different washing steps have to be correctly performed. The addition of the washing solution must be carried out with an efficient speed in order to create turbulences into the tubes.

Measure the remaining radioactivity bound to the tube with a gamma scintillation counter for 2 minutes.

9. QUALITY CONTROL

Good laboratory practices require that quality control samples be used in each series of assays to check the quality of the results obtained. All specimens should be treated identically, and result analysis using the appropriate statistical methods is recommended.

10. RESULTS

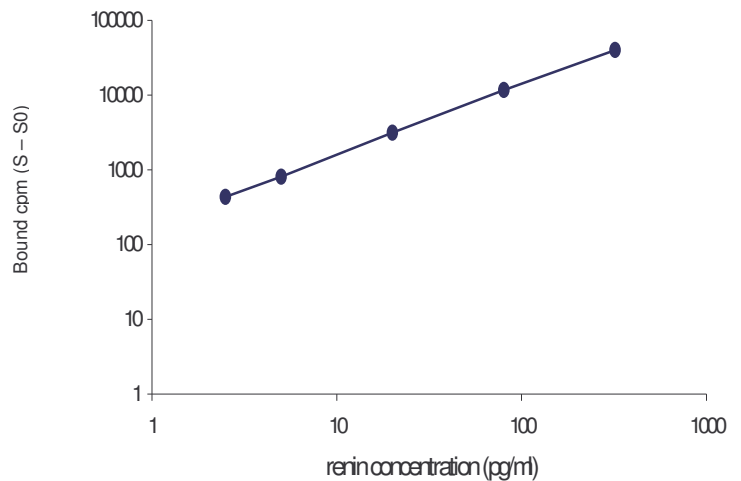
* Determine the mean value for counts of each duplicate (S – S₀). Express in counts per minute (or in B/T%) the bound activity of each standard, control and sample.

* It is possible to plot either net cpm or % B/T versus concentration on lin-log or log log paper graph. The most suitable form of graph representation for the standard curve is obtained by linear regression (the least squares method) (log-log).

* The concentration of each sample can be determined directly from the standard curve, as a function of the bound activity value.

Typical standard curve (example only) : these data must under no circumstances be substituted for results obtained in the laboratory.

	Bound cpm	Concentration pg/ml
Total activity	161814	
S0	57	0
S1	432	2.5
S2	804	5
S3	3133	20
S4	11700	80
S5	39943	320
Control sample C : 40 +/- 7 pg/ml	5613	39.2
Sample 1	1636	10.7
Sample 2	6725	47.3
Sample 3	17375	127



11. PROCEDURAL LIMITATIONS

Samples which show turbidity, haemolysis, hyperlipemia or contain fibrin may give misleading results.

Do not extrapolate sample values beyond the last standard. Dilute the samples concerned and re-assay.

12. EXPECTED VALUE

It is critically important to recognize that many factors (posture, age, sodium intake, menstrual cycle) can influence the active renin level. Thus, and with every diagnostic test, it is recommended that each laboratory establishes its normal range values according to its own population.

Study performed on 101 healthy patients (Male and Female from 20 to 80 years, without any cardiovascular treatment (beta blocker, anti inflammatory, antidiuretic,...) with 2 samplings per patient (1 after 1 hour rest and second after 1 hour walking) has given the following results :

RESULTS	AGE	N	MEAN (pg/ml)	LIMITS (pg/ml)
Upright	20-40	24	14.2	(5.1 – 38.7)
	40-60	41	15.4	(1.8 – 59.4)
	> 60	36	10.7	(0.4 – 33.3)
Supine	20-40	24	8.5	(3.6 – 20.1)
	40-60	41	6.3	(1.1 – 20.2)
	> 60	36	5.6	(0.1 – 16.1)

92 % of the values are between 3 and 33 pg/ml in an upright position

92 % of the values are between 3 and 16 pg/ml in a supine position

**13. SPECIFIC CHARACTERISTICS OF THE ASSAY****13.1. Imprecision**

Within-run reproducibility: 6 samples have been measured 10 times in the same assay with the following result.

Samples N°	Mean (pg/ml)	Standard deviation (pg/ml)	CV %
E1	7.31	0.33	4.5
E2	12.4	0.5	4.0
E3	21.2	0.6	2.8
E4	40.1	0.6	1.5
E5	127	0.8	0.6
E6	294	3.3	1.1

Between-run reproducibility: 5 samples have been measured (quadruplet) in 30 assays with 3 lots of tracer at different steps of validity, by 3 manipulators with the following result.

Samples N°	Mean (pg/ml)	Standard deviation (pg/ml)	CV %
A1	8.08	1.17	14.5
A2	11.4	0.85	7.4
A3	21.3	1.45	6.8
A4	122	3.24	2.7
A5	269	12.2	4.5

13.2. Accuracy**a) Recovery test**

Known quantities of renin were added to human sera. The recovery percentages of renin the samples ranged from 94 to 115 %. 3 human samples have been spiked with various active concentrations and have given the following result.

Samples N°	Obtained value (pg/ml)	Theoretical value (pg/ml)	Recovery
A6 (29.5)	4.7	5	94
A7 (53.4)	10.5	10	105
A8 (108)	23	20	115

(initial value)

b) Dilution test

High concentration samples were diluted. The recovery percentages obtained were between 94 and 106 %. 3 human samples have been diluted with S0 and have given the following result.

Dilution (initial value)	Obtained value (pg/ml)	Expected value (pg/ml)	Recovery %	
(119)	1/2	61	59.5	103
	1/5	25	23.8	105
	1/10	11.8	11.9	99
(164)	1/2	87	82	106
	1/5	34	32.8	105
	1/10	15.4	16.4	94
(320)	1/2	164	160	103
	1/5	65	64	102
	1/10	32.6	32	102

**13.3. Specificity**

The specificity of the assay is guaranteed by the use of two complementary monoclonal antibodies. Human renin is recognized. No interference was observed when samples were spiked with any of the following substances : pro-renin, cathepsin D (another enzyme of the aspartyl protease family), or various treatments for hypertension : Captopril, Renitec, Loxen, or Lasilix.

Substance spiked	Spiked concentration	Cross reactivity (%)
Pro-renin	400 ng/ml	0 à 1.8 *
Cathepsin D	0.5 unit/ml	< 0.001
Captopril	50 µg/ml	< 0.001
Renitec	50 µg/ml	< 0.001
Loxen	50 µg/ml	< 0.001
Lasilix	50 µg/ml	< 0.001

* This range is given for your guidance only, considering that absence of active renin (generated by auto or environmental activation), in prorenin preparation, can not be guaranteed. Therefore, this estimated cross reactivity may only represent the contamination of a prorenin preparation with active renin.

13.4. Detection limit

The detection limit is defined as being the smallest detectable concentration different from zero with a probability of 95 %. It has been found under **1 pg/ml**.

ASSAY FLOW CHART

Tubes	Standards (S0-S5) Controls (C) Samples µl	Tracer ¹²⁵ I (R2) µl	Mix gently ---- Cover with Parafilm® --- Incubate 3 h à 18-25°C under constant horizontal agitation ---- Aspirate.	Washing solution (R3) ml	Aspirate --- Wash 2 times	Count for 2 minutes
T	-	100		-		
Standards	300	100		2		
Controls Samples	300	100		2		