RENIN-IRMA

KIP1531

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Read entire protocol before use.

RENIN-IRMA

I. INTENDED USE

Immunoradiometric assay kit for the determination of active Renin in human EDTA plasma. For research use only, not for use in diagnostic procedures.

II. GENERAL INFORMATION

A. Proprietary name : DIAsource Renin-IRMA Kit

B. Catalog number : KIP1531 : 96 tests

C. Manufactured by : DIAsource ImmunoAssays S.A.
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III. BACKGROUND

Renin , a polypeptidic enzyme (MW~ 40000) (1) also known as angiotensinogenase, is a circulating protease secreted by juxtaglomerular cells in the juxtaglomerular apparatus of the kidneys in response to low blood volume or low body NaCL content.

Renin activates the renin-angiotensin system by cleaving angiotensinogen produced in the liver into angiotensin I (inactive) which is further converted into angiotensin II (active) in the vascular epithelium of the lung. Angiotensin II can cause vasoconstriction by stimulating the central nervous system , in addition it stimulates ADH (antidiuretic hormone) secretion and aldosterone secretion from the adrenal gland .(6)

Regulation of blood pressure and renal glomerular filtration control (2) are the most important functions of renin -angiotensin system .

Plasmatic concentration of renin is influenced by concentration of circulating angiotensinogen and subsequently the concentration of angiotensin II. High plasmatic levels of angiotensin II reduce renin secretion.(negative feed back)

Determination of renin plasma levels is useful in the study of hypertension and in the follow up of hypertensive subjects (3).

Plasmatic concentration of renin decreases in subjects with hypertension due to a primary hyperaldosteronism (4), contrary to renovascular hypertension (5) where concentrations of renin and aldosterone are both elevated.

IV. PRINCIPLES OF THE METHOD

The DIAsource Renin-IRMA is an immunoradiometric assay based on coated-tube. Mab1, the capture antibody, is attached to the lower and inner surface of the plastic tube. Calibrators or samples added to the tubes will at first show low affinity for Mab1. Addition of Mab2, the signal antibody labelled with ^{125}I, will complete the system and trigger the immunological reaction. After washing, the remaining radioactivity bound to the tube reflects the antigen concentration.

V. REAGENTS PROVIDED

Reagents	96 Test Kit	Colour Code	Reconstitution
Tubes coated with anti Renin (monoclonal antibody)	2 x 48	black	**Ready** for use
Ab | **^{125}I** Anti-Renin^{125}I (monoclonal antibody) in Phophate buffer with bovine serum, azide (<0.1%)	1 vial 10.5 ml 760 kBq	red	**Ready** for use
CAL | **N** Calibrators 0-6 in human serum and thymol. See exact value on vial labels.	7 vials lyophilised	yellow	**Add** 2 ml distilled water
WASH | **SOLN** | **CONC** Wash solution (Tween 20-NaCl)	1 vial 40 ml	green	**Dilute** 20 x with distilled water (use a magnetic stirrer).
CONTROL | **N** Controls - N = 1 or 2 in human serum and thymol	2 vials lyophilised	silver	**Add** 2 ml distilled water

Note: 1. Use the zero calibrator for sample dilution.

2. 1 pg of the calibrator preparation is equivalent to 2.2 +/- 0.2 µIU of NIBSC 68/356.

Values obtained in pg/ml must be multiplied by 2.2 to obtain results in µIU/ml or mIU/l.

VI. SUPPLIES NOT PROVIDED

The following material is required but not provided in the kit:

1. Distilled water
2. Pipettes for delivery of: 100 µl, 300 µl, and 2 ml (the use of accurate pipettes with disposable plastic tips is recommended)
3. Plastic tubes for total counts
4. Vortex mixer
5. Tube shaker (400 rpm)
6. Magnetic stirrer
7. 5 ml automatic syringe (Cornwall type) for washing
8. Aspiration system (optional)
9. Any gamma counter capable of measuring ^{125}I may be used (minimal yield 70%).

VII. REAGENT PREPARATION

A. Calibrators: Reconstitute the calibrators with 2 ml distilled water
! For a complete solubilisation : after reconstitution, let the vials 30 min on a shaker, then vortex them.

B. Controls: Reconstitute the controls with 2 ml distilled water .

C. Working Wash solution: Prepare an adequate volume of Working Wash solution by adding 19 volumes of distilled water to 1 volume of Wash Solution (20x). Use a magnetic stirrer to homogenize. Discard unused Working Wash solution at the end of the day.

VIII. STORAGE AND EXPIRATION DATING OF REAGENTS

- Before opening or reconstitution, all kits components are stable until the expiry date, indicated on the label, if kept at 2 to 8°C.
- After reconstitution, the calibrators and controls are unstable, use them immediately after reconstitution, freeze them immediately in aliquots and keep them at –20°C for maximum 6 weeks. They are stable after 1 freeze-thaw cycle .
- Avoid subsequent freeze-thaw cycles.
- Freshly prepared Working Wash solution should be used on the same day.
- After its first use, tracer is stable until expiry date, if kept in the original well-closed vial at 2 to 8°C.
- Alterations in physical appearance of kit reagents may indicate instability or deterioration.

IX. SPECIMEN COLLECTION AND PREPARATION

- Samples must be EDTA plasma.
- If the test is not run within 4 h., plasma should be aliquoted and stored at –20°C.
- Avoid subsequent freeze-thaw cycles.

X. PROCEDURE

A. Handling notes

Do not use the kit or components beyond expiry date.
Do not mix materials from different kit lots.
Bring all the reagents to room temperature prior to use.
Thoroughly mix all reagents and samples by gentle agitation or swirling.
Use a clean disposable pipette tip for addition of each different reagent and sample in order to avoid cross-contamination. High precision pipettes or automated pipetting equipment will improve the precision.
Respect the incubation times.
Prepare a calibration curve for each run, do not use data from previous runs.

B. Procedure

1. Label coated tubes in duplicate for each calibrator, sample and control. For the determination of total counts, label 2 normal tubes.
2. Briefly vortex calibrators, controls and samples and dispense 300 µl of each into the respective tubes.
3. Dispense 100 µl of ^{125}Iodine labelled anti Renin into each tube, including the uncoated tubes for total counts.
4. Incubate for 180 minutes at room temperature on a tube shaker (400 rpm).
5. Aspirate (or decant) the content of each tube (except total counts). Be sure that the plastic tip of the aspirator reaches the bottom of the coated tube in order to remove all the liquid.
6. Wash tubes with 2 ml Working Wash solution (except total counts) and aspirate (or decant). Avoid foaming during the addition of the Working Wash solution.
7. Wash tubes again with 2 ml Wash solution (except total counts) and aspirate (or decant).
8. Let the tubes stand upright for two minutes and aspirate the remaining drop of liquid.
9. Count tubes in a gamma counter for 60 seconds.

XI. CALCULATION OF RESULTS

1. Calculate the mean of duplicate determinations.
2. On log-log, semi logarithmic or linear graph paper plot the c.p.m. (ordinate) for each calibrator against the corresponding concentration of Renin (abscissa) and draw a calibration curve through the calibrator points, reject the obvious outliers.
3. Read the concentration for each control and sample by interpolation on the calibration curve.
4. Computer assisted data reduction will simplify these calculations. If automatic result processing is to be used, a 4-parameter logistic function curve fitting is recommended

XII. TYPICAL DATA

The following data are for illustration only and should never be used instead of the real time calibration curve.

Renin-IRMA		cpm	B/T (%)
Total count			100
Calibrator	0 pg/ml 0 µIU/ml	152	
	4 pg/ml 8.8 µIU/ml	579	0.14
	9 pg/ml 19.8 µIU/ml	984	0.27
	47 pg/ml 103.4 µIU/ml	3826	1.18
	95 pg/ml 209 µIU/ml	8161	2.58
	250 pg/ml 550 µIU/ml	20851	6.67
	520 pg/ml 1144 µIU/ml	55190	17.73

1 pg of the calibrator preparation is equivalent to 2.2 +/- 0.2 µIU of NIBSC 68/356

XIII. PERFORMANCE AND LIMITATIONS

A. Detection limit

Twelve zero calibrators were assayed along with a set of the other calibrators. The detection limit, defined as the apparent concentration of the average count at zero binding plus two standard deviations, was 0.78 pg/ml

B. Specificity

The Cross-reactivity of Prorenin in this Renin IRMA assay was determined by adding various concentrations of Prorenin to a plasma matrix and by measuring the apparent Renin response . Prorenin Cross-reactivity was found to be 0.3 % .

The potentially interfering effects of hemoglobin at 7.5 mg/ml and of bilirubin at 0.2 mg/ml have been evaluated. The results of this test (see the table below) show a decrease of approximatively 10% of plasma values. The recommendation is to avoid hemolized samples and bilirubin containing samples.

Sample tested	**Renin value (pg/ml)**	**+ Human Hb at 7.5 mg/ml (pg/ml)**
1.	21.2	18.5
2.	57.1	50
Sample tested	**Renin value (pg/ml)**	**+ bilirubine at 0.2 mg/ml (pg/ml)**
3..	22.6	20
4.	58.1	53.4

C. Precision

INTRA-ASSAY PRECISION

Sample	N	<X> ± SD (pg/ml)	CV (%)
A	10	20.2 ± 1.7	8.5
B	10	67.7 ± 2.0	3.0

INTER-ASSAY PRECISION

Sample	N	<X> ± SD (pg/ml)	CV (%)
A	6	15.5 ± 1.7	11
B	6	59.3 ± 2.4	4

SD: Standard Deviation; CV: Coefficient of variation

D. Accuracy

DILUTION TEST

Sample	Dilution	Theoretical Concent. (pg/ml)	Measured Concent. (pg/ml)
	1/4	258.6	
	1/8	129.3	125.5
	1/16	64.6	62.3
	1/32	32.3	29.4
	1/64	16.1	14.2
	1/128	8	6.7

Sample was diluted with zero calibrator
Value of undiluted sample : 1034 pg/ml

RECOVERY TEST

Added Renin (pg/ml)	Recovered Renin (pg/ml)	Recovery (%)
11.8	10.5	89
24.2	21.4	88
57	53	93
117	96	82

E. Hook effect

A sample spiked with human Renin up to 90 000 pg/ml gives a signal above the highest calibrator concentration .

F. Reference Intervals

Normal range has been determined on EDTA plasma of 60 apparently healthy subjects, fasting, aged from 20 to 60:40 males and 20 females, without oestrogen-progesterone treatment and without treatment for hypertension. For each subject, two blood samples were taken: one after 1 hour of activity in an upright position and another after remaining in a supine position for one hour.
Range limits are fixed from 5 to 95 percentile

Upright 1.3 - 13.8 pg /ml
Supine 1.0 - 8.2 pg /ml

Each laboratory should establish its own normal range of values.
Be careful many factors can influence renin levels (age, post ure, estrogen treatment, antihypertensive medication,…)

XIV. LIMITATIONS

- Specimens from subjects who have received preparations of mouse monoclonal antibodies for 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.
- Heterophilic antibodies in human serum can react with reagent immunoglobulins, interfering with in vitro immunoassays. *S*ubjects routinely exposed to animals or animal serum products can be prone to this interference and anomalous values may be observed in case of the presence of heterophelic antibodies. Carefully evaluate the results of subjects suspected of having these antibodies.

XV. INTERNAL QUALITY CONTROL

- If the results obtained for Control 1 and/or Control 2 are not within the range specified on the vial label, the results cannot be used unless a satisfactory explanation for the discrepancy has been given.
- If desirable, each laboratory can make its own pools of control samples, which should be kept frozen in aliquots.
- Acceptance criteria for the difference between the duplicate results of the samples should rely on Good Laboratory Practices.

XVI. PRECAUTIONS AND WARNINGS

Safety

For research use only, not for use in diagnostic procedures.
This kit contains ^{125}I (half-life: 60 days), emitting ionizing X (28 keV) and γ (35.5 keV) radiations.
This radioactive product can be transferred to and used only by authorized persons; purchase, storage, use and exchange of radioactive products are subject

to the legislation of the end user's country. In no case the product must be administered to humans or animals.
All radioactive handling should be executed in a designated area. away from regular passage. A logbook for receipt and storage of radioactive materials must be kept in the lab. Laboratory equipment and glassware, which could be contaminated with radioactive substances, should be segregated to prevent cross contamination of different radioisotopes.
Any radioactive spills must be cleaned immediately in accordance with the radiation safety procedures. The radioactive waste must be disposed of following the local regulations and guidelines of the authorities holding jurisdiction over the laboratory. Adherence to the basic rules of radiation safety provides adequate protection.
The human blood components included in this kit have been tested by Europe and approved and/or FDA approved methods and found negative for HbsAg, anti HCV, anti-HIV-1 and 2. No known method can offer complete assurance that human blood derivatives will not transmit hepatitis, AIDS or other infections. Therefore, handling of reagents, serum or plasma specimens should be in,accordance with the local safety procedures. All animal products and derivatives have been collected from healthy animals. Bovine components originate from countries where BSE has not been reported. Nevertheless, components containing animal substances should be treated as potentially infectious.
Avoid any skin contact with reagents (sodium azide as preservative). Azide in this kit may react with lead and copper in the plumbing and in this way form highly explosive metal azides. During the washing step, flush the drain with a large amount of water to prevent azide build-up.
Do not smoke, drink, eat or apply cosmetics in the working area. Do not pipette by mouth. Use protective clothing and disposable gloves.

XVII. BIBLIOGRAPHY

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(4) Screening of primary aldosteronism :Schirpenbach C. and al ; Best Pract. Res. Clin. Endocrinol. Metab.(2006) : 20(3) : 369-384 .
(5) Diagnostic procedure in renovascular hypertension :Distler A. and al. Clinical nephrology (1991) :36(4):174-180
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XVIII SUMMARY OF THE PROTOCOL

	TOTAL COUNTS µl	**CALIBRATORS CONTROLS µl**	**SAMPLE(S) µl**
INCUBATION			
Calibrators (0 to 6), controls	-	300	-
Samples	-	-	300
Tracer	100	100	100
Incubation	180 minutes at room temperature with shaking at 400 rpm		
Separation	-	Aspirate (or decant)	
Working Wash solution		2 ml	
Separation		Aspirate (or decant)	
Working Wash solution		2 ml	
Separation		Aspirate (or decant)	
Counting	Count tubes for 60 seconds		

DIAsource Catalogue Nr : KIP1531	P.I. Number : 1700473	Revision nr : 131029/1

	Used symbols
	Consult instructions for use
	Storage temperature
	Use by
LOT	Batch code
REF	Catalogue number
CONTROL	Control
IVD	In vitro diagnostic medical device
	Manufacturer
Σ	Contains sufficient for <n> tests
WASH SOLN CONC	Wash solution concentrated
CAL 0	Zero calibrator
CAL N	Calibrator #
CONTROL N	Control #
Ag 125I	Tracer
Ab 125I	Tracer
Ag 125I CONC	Tracer concentrated
Ab 125I CONC	Tracer concentrated
	Tubes
INC BUF	Incubation buffer
ACETONITRILE	Acetonitrile
SERUM	Serum
DIL SPE	Specimen diluent
DIL BUF	Dilution buffer
ANTISERUM	Antiserum
IMMUNOADSORBENT	Immunoadsorbent
DIL CAL	Calibrator diluent
REC SOLN	Reconstitution solution
PEG	Polyethylene glycol
EXTR SOLN	Extraction solution
ELU SOLN	Elution solution
GEL	Bond Elut Silica cartridges
PRE SOLN	Pre-treatment solution
NEUTR SOLN	Neutralization solution
TRACEUR BUF	Tracer buffer
	Microtiterplate
Ab HRP	HRP Conjugate
Ag HRP	HRP Conjugate
Ab HRP CONC	HRP Conjugate concentrate
Ag HRP CONC	HRP Conjugate concentrate
CONJ BUF	Conjugate buffer
CHROM TMB CONC	Chromogenic TMB concentrate
CHROM TMB	Chromogenic TMB solution
SUB BUF	Substrate buffer
STOP SOLN	Stop solution
INC SER	Incubation serum
BUF	Buffer
Ab AP	AP Conjugate
SUB PNPP	Substrate PNPP
BIOT CONJ CONC	Biotin conjugate concentrate
AVID HRP CONC	Avidine HRP concentrate
ASS BUF	Assay buffer
Ab BIOT	Biotin conjugate
Ab	Specific Antibody
SAV HRP CONC	Streptavidin HRP concentrate
NSB	Non-specific binding
2nd Ab	2nd Antibody
ACID BUF	Acidification Buffer
DIST	Distributor
TRAY	Incubation trays
PMSF	PMSF solution
	Protect from light
STRIP	Dot Strip
SUB	Substrate
EXTR SOLN CONC	Extraction Buffer Concentrate
CART	Cartridge
SAV HRP	Streptavidin HRP
PIPETTE	Pipette
WASH SOLN	Wash buffer