Angiotensin I RIA

KIRB3518

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History

Summary of change :

Previous Version : 230421	**Current Version :** 251215
2. REAGENTS PROVIDED **^{125}I-labeled angiotensin I: one 11 ml vial** (ready-to use) The vial contains 260 kBq, at the date of manufacture, of 125I-labeled angiotensin I in buffer with bovine serum albumin and a dye.	**2. REAGENTS PROVIDED** **^{125}I-labeled angiotensin I: one 11 mL vial** (ready-to-use) The vial contains 260 kBq, at the date of manufacture, of 125 I-labeled angiotensin I in buffer with bovine serum albumin and a dye, and sodium azide (<0.1%).

Angiotensin I RIA

en

Radioimmunoassay of Angiotensin I for the in vitro determination of Plasma Renin Activity (PRA) in human plasma

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For Research Use Only

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1. PRINCIPLE OF THE ASSAY

The Angiotensin I RIA KIT serves for the quantitative determination of plasma renin activity (PRA) by the radioimmunoassay of the product of the reaction, angiotensin I.
The generation of angiotensin I is the result of the enzymatic cleavage of the renin substrate, angiotensinogen, in plasma samples in the presence of ACE inhibitor (ACE - Angiotensin-Converting Enzyme), an enzymatic inhibitor that blocks the conversion of angiotensin I to angiotensin II.
The radioimmunoassay of angiotensin I is a competition assay. Samples and calibrators are incubated with 125I-labeled angiotensin I, as a tracer, in polyclonal antibody-coated tubes. After incubation, the contents of the tubes are rinsed so as to remove unbound 125I-labeled tracer. The bound radioactivity is then determined in a gamma counter. The angiotensin I concentrations in the samples are obtained by interpolation from the standard curve. The concentration of angiotensin I in the samples is indirectly proportional to the radioactivity.

2. REAGENTS PROVIDED

All reagents of the kit are stable until the expiry date indicated on the kit label, if stored at 2-8°C. Storage conditions for reagents after reconstitution or dilution are indicated in paragraph Assay Procedure. Expiry dates printed on component vial labels apply to the long-term storage by manufacturer only, prior to assembling of the kit. Do not take into account.

Anti-angiotensin polyclonal antibody-coated tubes: 2 x 50 tubes (ready-to-use)

Ag	125I

^{125}I-labeled angiotensin I: one 11 ml vial (ready-to use)
The vial contains 260 kBq, at the date of manufacture, of 125I-labeled angiotensin I in buffer with bovine serum albumin and a dye, and sodium azide (<0.1%).

CAL	N

Calibrators: 6 x 1 ml vials (ready-to-use)
The calibrator vials contain from 0 to approximately 30 ng/mL of angiotensin I in buffer with bovine serum albumin and sodium azide (<0.1%; see § Precautions). The exact concentration is indicated on each vial label. The calibrators are traceable to the international standard 86/536.

CONTROL

Control sample: one 1 ml vial (ready-to-use)
The vial contains angiotensin I in buffer with bovine serum albumin and sodium azide (<0.1%; see § Precautions). The concentration range is indicated on a supplement. The control sample is traceable to the international standard 86/536.

NEUTR	SOLN

Enzymatic inhibitor; one vial (lyophilized)
Inhibitor contains sodium azide (<0.1%, see § Precautions)

WASH	SOLN	CONC

Wash Solution (20x): one 50 ml vial
Concentrated solution has to be diluted before use

3. MATERIAL REQUIRED BUT NOT PROVIDED

In addition to standard laboratory equipment, the following items are required:

- Precision micropipette (75 µL).
- Semi-automatic pipette (100 µL, 200 µL, 2 mL).
- Water bath.
- Ice bath.
- Vortex type mixer.
- Horizontal or orbital shaker.
- Aspiration system.
- Gamma counter set for ^{125}I.

4. PRECAUTIONS

4.1 General remarks:

- Enzymatic inhibitor solution, calibrators, control sample and analyzed samples must be cooled to 2-8°C before pipeting.
- The vials with calibrators and controls should be opened as shortly as possible to avoid excessive evaporation.
- Do not mix the reagents from kits of different lots.
- A standard curve must be established with each assay.
- It is recommended to perform the immunoassay in duplicate.
- Each tube must be used only once.

4.2 Basic rules of radiation safety

The purchase, possession, utilization, and transfer of radioactive material is subject to the regulations of the country of use.
Adherence to the basic rules of radiation safety should provide adequate protection:

- No eating, drinking, smoking or application of cosmetics should be carried out in the presence of radioactive materials.
- No pipetting of radioactive solutions by mouth.
- Avoid all contact with radioactive materials by using gloves and laboratory overalls.
- All manipulation of radioactive substances should be done in an appropriate place, distant from corridors and other busy places.
- Radioactive materials should be stored in the container provided in a designated area.
- A record of receipt and storage of all radioactive products should be kept up to date.
- Laboratory equipment and glassware which are subject to contamination should be segregated to prevent cross-contamination of different radioisotopes.
- Each case of radioactive contamination or loss of radioactive material should be resolved according to established procedures.
- Radioactive waste should be handled according to the rules established in the country of use.

4.3 Sodium azide

Some reagents contain sodium azide as a preservative. Sodium azide can react with lead, copper or brass to form explosive metal azides. Sodium azide disposal must be in accordance with appropriate local regulations.

4.4 ProClin 300

R43 may cause sensitisation by skin contact

4.5 Materials of human origin

The materials of human origin, contained in this kit, were found negative for the presence of antibodies to HIV 1 and HIV 2, antibodies to HCV, as well as of Hepatitis B surface antigen (HBsAg). However, they should be handled as if capable of transmitting disease. No known test method can offer total assurance that no virus is present. Handle this kit with all necessary precautions.
All specimens should be handled as potentially infectious and waste should be discarded according to the country rules.

5. SPECIMEN COLLECTION, PROCESSING, STORAGE AND DILUTION

- Plasma samples have to be collected into cold EDTA tubes
- Separate plasma from cells by centrifugation at 2-8°C.
- Keep plasma samples frozen (<-20°C, 1 year maximum) if determination is not to be performed immediately, after aliquoting in order to avoid repeated freezing and thawing.

Note: The temperature of plasma samples must be kept at 2-8°C in the course of sampling. Avoid further manipulation to prevent both formation and decomposition of angiotensin I.
- If samples have concentrations greater than the highest calibrator, they must be diluted into the zero calibrator.

6. ASSAY PROCEDURE

6.1 Preparation and storage of reagents

6.1.1 Preparation of enzymatic inhibitor solution
The content of the vials is reconstituted with the volume of cold distilled water (4°C) indicated on the label and mixed. The reconstituted enzymatic inhibitor may be stored at 2-8°C until the expiry date of the kit. Occasional presence of turbidity in Inhibitor after reconstitution does not affect assay performance.

6.1.2. Preparation of wash solution
Pour the content of the vial into 950 mL of distilled water and homogenize. The diluted solution may be stored at 2-8°C until the expiry date of the kit.

6.2 Enzymatic step – generation of Angiotensin I

6.2.1 Remarks and recommendations
- The enzymatic inhibitor has to be cooled to 4°C before addition to the sample.
- Both incubation temperatures (4°C and 37°C) must be adhered to strictly, even slight variations may cause severe errors in determination.
- The enzymatic incubation time at 37°C should be determined as precisely as possible and kept within narrow limits for the whole set of tubes.
- The promptness of the temperature increase from 4°C to 37°C and the following reverse drop are critical. A circulating water bath is convenient for warming and, the use of an iced-cooled water bath is advisable for cooling.
- The promptness of the temperature increase and drop may be improved by using tubes made of material with good thermal conductivity (glass).
- If low plasma renin activity of the sample is expected, the incubation time of the enzymatic step may be prolonged for up to 3 hours.

6.2.2 Enzymatic step – procedure

Attention: Do not treat the calibrators and the control sample.

- Add 200 µL of pre-cooled enzymatic inhibitor to 200 µL of each plasma sample and mix.
- Split each sample into two 200 µL aliquots.
- Place the first aliquot into an ice-cold water bath in a refrigerator (intended for the determination of background angiotensin I at 4°C).
- Place the second one into the water bath set for 37°C (intended for the determination of generated angiotensin I at 37°C).
- Incubate all aliquots for 1 hour.

- **After incubation, cool samples from 37°C to 4°C rapidly using ice water bath**

6.3 Immunoassay procedure

Step 1 Additions *	Step 2 Incubation	Step 3 Counting
To antibody coated tubes, add successively: - 75 µL of calibrator, control or sample after enzymatic incubation at 37°C and at 4°C respectively and - 100 µL of tracer.** Vortex gently 1-2 seconds.	Incubate 2 hours at 18-25°C with shaking (> 280 rpm).	Aspirate carefully the contents of tubes (except the 2 tubes "total cpm"). Wash with 2 mL of wash solution. Aspirate twice. Determine activity (cpm) for 1 min.

* Calibrators, control sample and analyzed samples have to be cooled to 4°C before pipeting. Mix samples gently before they are added.
** Add 100 µL of tracer to 2 additional tubes to obtain total cpm.

7. RESULTS

Results are obtained from the calibration curve by interpolation. The curve serves for the determination of angiotensin I concentrations in samples measured at the same time as the calibrators.

7.1 Calibration curve

The results in the quality control department were calculated using cubic regression curve fit with logit of B/T or B/B0 on the vertical axis and log of analyte concentration of the calibrators on the horizontal axis.

Other calculation methods may give slightly different results.

Total activity : 68 511 cpm				
Calibrators	**Angiotensin I (ng/mL)**	**cpm (n=3)**	**B/T (%)**	**B/B0 (%)**
0	0.00	17 444	25.5	100
1	0.30	13 732	20.0	78.7
2	1.00	9 390	13.7	53.8
3	3.00	5 431	7.93	31.1
4	10.0	2 746	4.01	15.7
5	30.0	1 243	1.81	7.13

(Example of calibration curve, do not use for calculation)

7.2 Samples

For the control and samples incubated at 4°C, or at 37°C, locate the B/T (%) or the B/B0 (%) value on the vertical axis and read off the corresponding angiotensin I concentration in ng/mL on the horizontal axis.

7.3 Calculation of plasma renin activity

The determination of plasma renin activity is performed indirectly by the measurement of the in vitro generation of angiotensin I (A-I) per hour. Background A-I, determined on plasma samples incubated at 4°C, is subtracted from the A-I generated at 37°C for the calculation of PRA using the following equation:

$$\text{PRA ng of A-I /mL/hr} = \frac{[\text{A-I (37°C)} - \text{A-I (4°C)}] \times 2}{\text{Enzymatic incubation time (hrs)}}$$

Where

A-I (37°C): angiotensin concentration in ng/mL of sample incubated at 37°C
A-I (4°C) : angiotensin concentration in ng/mL of sample incubated at 4°C

8. QUALITY CONTROL

Good laboratory practices imply that control samples must be used regularly to ensure the quality of the results obtained. These samples must be processed exactly in the same way as the assay samples, and it is recommended to analyse their results using appropriate statistical methods.
Failure to obtain the appropriate values for controls may indicate imprecise manipulations, improper sample handling or deterioration of reagents.

In case of packaging deterioration or if data obtained show some performance alteration, please contact your local distributor or use the following e-mail address:

products.support@diasource.be

9. PERFORMANCE CHARACTERISTICS

(For more details, see the data sheet "APPENDIX")
Representative data are provided for illustration only. Performance obtained in individual laboratories may vary

9.1 Sensitivity

9.1.1 Analytical sensitivity: 0.07 ng/mL

9.1.2 Functional sensitivity: 0.20ng/mL

9.2 Specificity

The antibody used in the immunoassay is highly specific for angiotensin I. Extremely low cross reactivity was obtained against several molecules.
Moreover, the influence of possible interferences on PRA result is eliminated by subtraction of background.

9.3 Precision

9.3.1 Intra-assay
Samples were assayed in at least 25 replicates in the same series. The coefficients of variation were found below or equal to 11.3%.

9.3.2 Inter-assay
Samples were assayed in duplicate in 10 different series. Coefficients of variation were found below or equal to 20.9%.

9.4 Accuracy

9.4.1 Dependence on time of enzymatic incubation
The samples were incubated with enzymatic inhibitor for 60, 120, and 180 minutes. No significant effect on PRA results was found.

9.4.2 Dilution test
High-concentration plasma samples were serially diluted in the zero calibrator. The recovery percentages obtained were between 78.2% and 98.8%.

9.4.3 Recovery test

Low-concentration plasma samples were spiked with known quantities of angiotensin I. The recovery percentages were obtained between 104% and 123%.

9.5 Measurement range (from analytical sensitivity to highest calibrator):
0.07 to approximatively 30 ng/mL.

10. LIMITATIONS OF THE METHOD

Failure to follow these instructions for use (IFU) may significantly affect results.
Do not use hemolyzed, lipemic or icteric samples. For more details, see Appendix, § Interference.
In immunoassays, the possibility exists for interference by heterophile antibodies in the sample. Specimen who have been regularly exposed to animals or have received immunotherapy or diagnostic procedures utilizing immunoglobulins or immunoglobulin fragments may produce antibodies, e.g. HAMA, that interfere with immunoassays. Immunoassays may be also affected by presence of anti-avidin or anti-streptavidin antibodies, as well as by the presence of autoantibodies directed against the determined analyte. Such interfering antibodies may cause erroneous results.

11. BIBLIOGRAPHY

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Other translations of this instructions for use can be downloaded from our website: https://www.diasource-diagnostics.com/

APPENDIX

PERFORMANCE CHARACTERISTICS

Representative data are provided for illustration only. Performance obtained in individual laboratories may vary.

Interference

Plasma samples containing angiotensin I concentrations (low and high) were spiked with multiple concentrations of the substances listed below and assayed using Angiotensin I RIA KIT. Values were calculated as described in CLSI EP07, 3rd ed. [6]. Interference was determined by testing controls (no interfering substance added) and matched test samples (with interfering substance added). No interference (defined as a shift in dose > 15 %) was found for addition of interferent up to concentration stated in the table below.

Interferent	Test concentration
Biotin	1,631 NG/ML
Conjugated bilirubin	474.4 µG/ML
Hemoglobin	10,044 µG/ML
Triglycerides	15.20 MG/ML
Unconjugated bilirubin	384.3 µG/ML

In spite of hemoglobin, bilirubin (conjugated, unconjugated) and triglyceride interference data in the table, we advise to avoid using hemolyzed, lipemic or icteric samples.

Specificity

Analogue	Cross-reactivity (%)
Angiotensin I	100
Angiotensin II	ND
Angiotensin III	ND
Tetradecapeptide	ND
Angiotensinogen	ND

ND = Non-detectable

Precision

Intra-assay

Sample	P1	P2
Number of determinations	25	25
Pra, ng/ml/hour	1.49	2.94
Cv (%)	11.25	11.25

Inter-assay

After generation of Angiotensin I, samples were determined in duplicates in 10 different series according to the procedure of the kit. Plasma renin activity was obtained and used for the calculation of inter-assay precision.

Sample	P1	P2	P3	P4	P5
Number of determinations	10	10	10	10	10
Pra, ng/ml/hour	0.69	3.37	7.08	15.09	24.15
Cv (%)	20.9	9.57	8.72	9.74	11.8

Accuracy

Dependence on time of enzymatic incubation

No dependence of PRA on time was observed.

Time, minutes		60	120	180
Pra Ng/ml/hour	Sample 1	0.30	0.32	0.33
	Sample 2	0.56	0.48	0.53
	Sample 3	1.04	1.15	1.19
	Sample 4	1.64	1.56	1.65
	Sample 5	5.12	5.19	4.93

Dilution test

Samples were diluted in zero calibrator and assayed according to the assay procedure of the kit.

Sample	Dilution Factor	Theoretical conc. (NG/ML)	Observed conc. (NG/ML)	Recovery (%)
P1	-	-	2.45	-
	1:2	1.23	1.21	98.78
	1:4	0.61	0.56	91.43
	1:8	0.31	0.27	88.16
P2	-	-	3.05	-
	1:2	1.53	1.46	95.74
	1:4	0.76	0.64	83.93
	1:8	0.38	0.34	89.18
P3	-	-	9.21	-
	1:2	4.61	4.30	93.38
	1:4	2.30	1.82	79.04
	1:8	1.15	0.90	78.18
	1:16	0.58	0.45	78.18

Recovery test

Plasma samples were spiked with known quantities of angiotensin I and assayed according to the procedure of the kit.

Initial conc. (ng/ml)	Added angiotensin (ng/ml)	Observed conc. (ng/ml)	Observed addition (ng/ml)	Recovery (%)
0.88	1.01	1.89	2.09	110.7
	1.30	2.18	2.68	122.9
	1.93	2.81	3.20	113.9
1.39	1.01	2.40	2.64	110.2
	1.30	2.69	2.98	110.8
	1.93	3.32	3.59	108.2
1.54	1.01	2.54	2.84	111.7
	1.30	2.84	3.31	116.7
	1.93	3.46	4.03	116.4
2.60	1.01	3.61	3.83	106.2
	1.30	3.90	4.59	117.7
	1.93	4.53	5.20	114.9
2.62	1.01	3.62	3.75	103.5
	1.30	3.92	4.19	107.0
	1.93	4.55	4.82	106.0

Expected data for children

Results are sorted according to age.

Children		Angiotensin (ng/ml/hr)				
Upright	N	Min	Max	Median	2.5th percentile	97.5th percentile
2-9 years	27	0.60	7.38	3.20	0.76	6.64
10-15 years	16	0.58	3.98	1.52	0.64	3.93

125I Characteristics

T1/2 (125I) = 1443 h = 60.14 d

^{125}I	E (MeV)	%
γ	0.035	6.5
K_α X-RAY	0.027	112.5
K_β X-RAY	0.031	25.4