Angiotensin II -RIA

RB320RUO

DIAsource ImmunoAssays S.A. - Rue du Bosquet, 2 - B - 1348 Louvain-la-Neuve – Belgium

History

Summary of change:

Previous Version: **200224-1**	**Current Version:** **200701**
	Text added: **V. REAGENTS PROVIDED** CAL and CONTROL N see exact value on vial label
Room temperature	Room temperature (18-25°C)
X. PROCEDURE B. Procedure and **XVIII. SUMMARY OF THE PROTOCOL:** Incubation at 4°C	**X. PROCEDURE C. Assay Procedure** and **XVIII. SUMMARY OF THE PROTOCOL:** Replacement by "incubation at 2-8°C"
	Text added: **XVI. PRECAUTIONS AND WARNINGS** For more information, see Material Safety Data sheet (MSDS).
Use of : - "calibrators" and "standards" terms - "standard curve"	Uniformization into : - "calibrator" - "calibration curve"
X. PROCEDURE **A. Extraction procedure of plasma** 8. Centrifuge all extraction tubes at 2000 g. for 15 minutes at 2-8°C.	**X. PROCEDURE** **A. Extraction procedure of plasma** 8. Centrifuge all extraction tubes at 2000 g. for 15 minutes at 4°C.
X. PROCEDURE **C. Assay Procedure** 11. Centrifuge all tubes for 15 minutes at 1700 g at 4° C or room temperature (18-25°C).	**X. PROCEDURE** **A. Extraction procedure of plasma** Addition of "codes" for the Recovery tubes : • Recovery tubes = (R) • Total Recovery tube = (TR) **C. Assay Procedure** 11. Centrifuge all tubes for 15 minutes at 1700 g at 4°C.
III. CLINICAL BACKGROUND	**III. BACKGROUND**
	XI. CALCULATION OF RESULTS Addition of a example for the correction of the plasma value after extraction
XIV. REFERENCE INTERVALS Each laboratory should establish its own normal range of expected values. Blood samples were drawn from 11 apparently healthy adults (09.00 - 10.00 a.m.) and Angiotensin II levels were determined. Observed Range: 19 - 38 pmol/L	Section XIV removed

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Read entire protocol before use.

Angiotensin II – RIA

I. INTENDED USE

Radioimmunoassay for the *in vitro* quantitative measurement of Angiotensin II in plasma
For Research use only. Not for use in diagnostic procedures.

II. GENERAL INFORMATION

A. Proprietary name : DIAsource Angiotensin II-RIA

B. Catalog number : RB320RUO : 100 tests

C. Manufactured by : DIAsource ImmunoAssays S.A.
Rue du Bosquet, 2, B-1348 Louvain-la-Neuve, Belgium.

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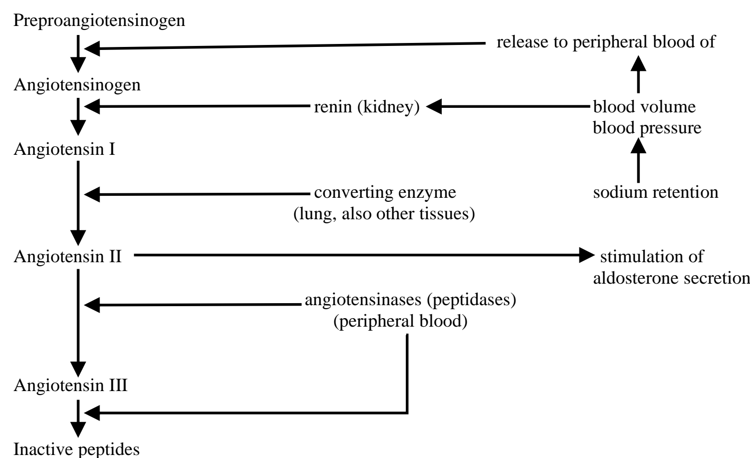
III. BACKGROUND

1. Biological activities

Angiotensin II is the biologically active product of the renin-angiotensin system (1,2).
The octapeptide angiotensin II (molecular weight 1046) is the strongest physiological vasoconstrictor known. From a large protein precursor (pre-proangiotensinogen) synthesized in the liver, it is liberated in a series of proteolytic steps catalysed by enzymes from various tissues (1, 2 4). Angiotensin II is very short-lived in the plasma: Once generated from angiotensin I, it is degraded further into physiologically inactive peptides by various plasma peptidases, at a plasma half-life of less than a minute (5). The scheme below gives an outline of the so-called renin-angiotensin system:



IV. PRINCIPLES OF THE METHOD

After extraction of the plasma samples, angiotensin II is assayed by a competitive radioimmunoassay. This radioimmunoassay is using a rabbit anti-angiotensin II antiserum and a radio-iodinated angiotensin II tracer. Bound and free phases are separated by a second antibody bound to solid phase particles, followed by a centrifugation step. The radioactivity in the bound fractions is measured and a typical calibration curve can be generated.
For professional use within a laboratory. The result shall not be used for clinical diagnosis or specimen management.

V. REAGENTS PROVIDED

Reagents	100 Tests Kit	Colour Code	Reconstitution
Ab Antiserum: Rabbit anti-angiotensin II antiserum	1 vial Lyophilised	Blue	**Add** 22 mL distilled water
Ag ^{125}I TRACER: ^{125}Iodine labelled Angiotensin II in phosphate buffer with human serum albumin and NaN_3.	1 vial Lyophilised 56 kBq	Red	**Add** 25 mL distilled water
DASP Double antibody solid phase: Goat anti-rabbit Ig's bound to solid phase in phosphate buffer with human serum albumin, Tween and sodium azide. (<0.1%).	1 vial 11 mL	Green	**Ready** for use
ASS BUF Assay buffer : phosphate buffer containing human serum albumin and sodium azide, (<0.1%).	2 vials 50 mL	Black	**Ready** for use
CAL Angiotensin II Calibrator, 300 pmol/L. **(see exact value on vial label)**	1 vial Lyophilised	Yellow	**Reconstitute** with distilled water by the volume stated on vial label**.**
CONTROL N Controls – N = 1 or 2 **(see exact value on vial label)**	2 vials Lyophilised	Silver	**Add** 2 mL distilled water

VI. SUPPLIES NOT PROVIDED

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

1. Pipettes (100 µL, 200 µL, 1 mL, 2 mL, 5 mL).
2. Repeating dispensers (100 µL, 200µL).
3. Measuring cylinder 25 mL.
4. Polystyrene tubes, polypropylene or glass-tubes.
5. Vortex.
6. Refrigerated centrifuge.
7. Ethanol p.A. 98%.
8. Vac-concentrator or N_2 (nitrogen).
9. Ice bath

VII. REAGENT PREPARATION

PREPARE ALL REAGENTS 15 MINUTES BEFORE USE !

A. **Antiserum:** Reconstitute with 22 mL distilled water. Mix gently. Stable at -20° C for at least 3 months after reconstitution
B. **^{125}I-angiotensin II :** Reconstitute with 25 mL distilled water. Mix gently. Stable at -20° C until expiry date.
C. **Double antibody solid phase :** Ready for use. The separation reagent should be placed on a magnetic stirrer for 10 minutes. It is possible to pipette the reagent with a repeating dispenser. Stable at 2-8° C.
D. **Assay buffer :** Ready for use. Stable at 2-8° C until expiry date.
E. **Angiotensin II calibrator 300 pmol/L:** Reconstitute with distilled water by the volume stated on vial label. Mix gently. Stable at -20°C for at least 3 months after reconstitution. Refer to table in section X. B. for the calibration curve preparation.
F. **Controls :** Reconstitute each vial with 2 mL distilled water. Stable at -20° C for at least 3 months after reconstitution. The concentration of the control is found on the label of the vials (without extraction).

VIII. STORAGE AND EXPIRATION DATING OF REAGENTS

This kit is stable until the stated expiry date if stored as specified.
Upon receipt of the kit, all reagents should be stored at 2-8°C.
The reconstituted reagents should be stored according to section VII.
The reconstituted reagents are stable according to section VII, but no longer than the expiry date.

IX. SPECIMEN COLLECTION

Careful standardization of the specimen preparation and sampling conditions is recommended. Due to the extreme lability of angiotensin II in biological fluid, much care must be taken to ensure that the blood sample is collected properly:

- draw blood from fasting specimen in recumbent position into cold tube containing EDTA;
- centrifuge immediately at 4°C to separate the plasma;
- freeze the sample immediately in plastic tubes at -20°C until assayed.

X. PROCEDURE

A. Extraction procedure of plasma

1. Label one extraction tube for each specimen sample. Label one additional tube (R) in order to estimate the extraction recovery.
2. Place the extraction tubes and ethanol on ice.
3. Pipette 1 mL of each sample into the appropriately labelled extraction tubes.
   DO NOT EXTRACT CALIBRATORS AND CONTROLS.
4. Prepare a recovery estimation tube (R) :
   - Pipette 1 mL of a random plasma sample into the recovery tube (R). The sample used for this recovery assay should have a protein matrix similar to the samples being tested.
   - Add 200 µL ^{125}I-angiotensin II tracer into the tube (R).
   - Extract this sample along with samples in step 6.
5. Prepare Total Recovery tubes (TR) :
   - Pipette 200 µL ^{125}I-angiotensin II tracer into two tubes (TR).
   - Add 200 µL assay buffer and mix.
   - Cap and set aside these tubes to be counted for recovery calculation.
6. Add 4 mL chilled ethanol to each sample and Recovery tube (R).
7. Mix and vortex for 2 minutes.
8. Centrifuge all extraction tubes at 2000 g. for 15 minutes at 4°C.
9. Decant supernatant from each extraction tube into previous prepared clean, appropriately labelled 16 x 100 mm tubes.
10. Evaporate the supernatants under a stream of nitrogen to dryness (at max. 37°C).
11. Reconstitute the dried samples by adding 1 mL assay buffer and vortex thoroughly.
12. Proceed RIA procedure immediately or store the extracted samples at -20°C up to two weeks before using it in the assay.
13. Reconstitute the dried recovery sample (R) by adding 1 mL assay buffer and vortex thoroughly.
14. Pipette 400 µL of the reconstituted recovery sample tube (R) into two 12 x 75 mm tubes.
15. Count the total recovery (TR) and recovery (R) tubes for at least two minutes in a gamma counter.

Recovery calculation:
Calculate % recovery by dividing the cpm in the recovery tubes (R) by cpm in the total recovery tubes (TR) and multiply by 1.0/0.4:

$$\% \text{ Recovery} : \frac{\text{cpm recovery tube (R)}}{\text{cpm total recovery tube (TR)}} \; \frac{1.0}{0.4} \times 100$$

B. Preparation of Calibrator solutions

Dilution	Angiotensin II Calibrator	Concentration 300 pmol/L
1000 µL of Angio II Calibrator + 1000 µL assay buffer. Vortex	Calibrator a	150 pmol/L
1000 µL of Calibrator a + 1000 µL assay buffer. Vortex	Calibrator b	75 pmol/L

1000 µL of Calibrator b + 1000 µL assay buffer. Vortex	Calibrator c	37.5 pmol/L
1000 µL of Calibrator c + 1000 µL assay buffer. Vortex	Calibrator d	18.8 pmol/L
1000 µL of Calibrator d + 1000 µL assay buffer. Vortex	Calibrator e	9.4 pmol/L
1000 µL of Calibrator e + 1000 µL assay buffer. Vortex	Calibrator f	4.7 pmol/L

C. Assay Procedure

1. Keep assay tubes and reagents in an ice bath during all pipetting steps.
2. Pipette 400 µL of each Calibrator, 400 µL of controls and 400 µL of each plasma extract in duplicate into the corresponding labelled polystyrene tubes.
3. Add 400 µL of assay buffer to the max. binding tubes (0 pmol/L).
4. Add 600 µL of assay buffer to the NSB (blank) tubes.
5. Add 200 µL of angiotensin II antiserum to each tube, except blank and TC-tubes.
6. Vortex and incubate for 6 hours at 2-8°C.
7. Add 200 µL of ^{125}I-Angiotensin II tracer to all tubes.
8. Vortex all tubes and incubate at 2-8° C for 18-22 hours.
9. While stirring continuously add 100 µL of the double antibody solid phase to all tubes, except TC- tubes.
10. Vortex and incubate 30-60 minutes. at 2-8° C.
11. Centrifuge all tubes for 15 minutes at 1700 g at 4° C.
12. Decant the supernatants carefully.
13. Count residue for 1-2 minutes.

XI. CALCULATION OF RESULTS

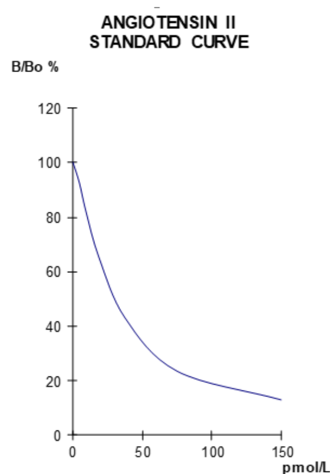
1. Subtract the mean count rate (cpm) of the NSB from the mean count rate (cpm) of the replicates of Calibrators, controls and specimen samples.
2. A calibration curve can be generated by plotting cpm, % B/Bo or %B/T of precipitated bound fraction, against the concentration of the angiotensin II Calibrators.
3. To obtain the angiotensin II concentration in the extracted specimen samples and controls, their cpm, % B/Bo or B/T of precipitated bound fractions are interpolated now from generated calibration curve.
4. The calibration curve can also be constructed by computer methods. For automated data reduction, both logit/log and Spline methods can be used.
5. Correct plasma values for % extraction recovery.

For example:
Patient sample concentration measured from the curve: 20 pmol/L
Extraction recovery measured after extraction : 65%
Patient sample concentration corrected : $\frac{20 \times 100}{65}$ = 30.8 pmol/L

Calibration Curve Data

	Average cpm	Corrected cpm	% B/Bo	Results (pmol/L)
Total counts	18582			
NSB	678			
Calibrator 0 pmol/L	9559	8881	100	
Calibrator f 4.7 pmol/L	8880	8202	92.4	
Calibrator e 9.4 pmol/L	7957	7279	82.0	
Calibrator d 18.8 pmol/L	7039	5781	65.1	
Calibrator c 37.5 pmol/L	4508	3830	43.1	
Calibrator b 75 pmol/L	2770	2099	23.6	
Calibrator a 150 pmol/L	1846	1168	13.1	
Control low	7359	6681	75.2	13.1
Control high	3145	2467	27.8	63.1

Example of Calibration Curve



XIII. PERFORMANCE AND LIMITATIONS

A. Sensitivity

The sensitivity judged as 3 standard deviations change from zero calibrator is 2.0 pmol/L.

B. Precision

Within-run					Between-run				
	n	Mean pmol/L	SD	% CV		n	Mean pmol/L	SD	% CV
sample A	20	13.3	0.44	3.3	sample A	6	11.6	0.55	4.8
sample B	20	64.9	1.97	3.0	sample B	6	60.9	2.4	3.9

C. Accuracy

Recovery
Four different samples are spiked with different amounts of angiotensin II Calibrator

Sample	Expected conc. (pmol/L)	Observed conc. (pmol/L)	% Recovery
A1	12.4	12.3	99.2
A2	23.9	23.5	96.8
A3	27.2	22.0	103.0
A4	46.0	51.1	111.0

D. Specificity

Angiotensin II antiserum is raised in rabbits. The following cross-reactivities were measured at 50% B/Bo.

Peptide	Cross-reaction
Angiotensin II	100
Angiotensin I	<0.1
Leu-Heptapeptide	100
Asn^{1}-Val^{5} Angiotensin II	30
Sar^{1} Ile^{8} Angiotensin II	100
Angiotensin III	80

E. Interference

Samples displaying cloudiness, haemolysis, hyperlipemia or containing fibrin may give inaccurate results.

XIV. INTERNAL QUALITY CONTROL

Controls should be carried out in each assay run. Two controls are included in the kit, the value (without extraction procedure) is indicated on the labels of the vials. Use controls as recommended by the control plasma manufacturer and in accordance with reference laboratories practice to monitor the accuracy and precision of reagents and techniques.

XV. PRECAUTIONS AND WARNINGS

Safety

For in vitro diagnostic use only. Not for use in diagnostic procedures.
Materials derived from human blood and used in the preparation of this kit were tested and found negative for hepatitis B surface antigen (HBsAg), antibodies to HCV and for antibodies to HIV-1 and HIV-2. However, handle all components as a possible source of infection.
The reagents in this kit contain sodium azide. Contact with copper or lead drain pipes may result in the cumulative formation of highly explosive azide deposits. On disposal of the reagents in the sewerage, always flush with copious amounts of water, which prevents metallic azide formation. Plumbling suspected of being contaminated with these explosive deposits should be cleaned thoroughly with 10% sodium hydroxide solution.
This kit contains ^{125}I (half-life: 60 days), emitting ionizing X (28 keV) and γ (35.5 keV) radiations. The radioactive material included may be received, acquired, possessed and used only by physicians, clinical laboratories or hospitals for in-vitro clinical or laboratory tests not involving internal or external administration of the material, or the radiation therefrom, to human beings or animals. Its receipt, acquisition, possession, use and transfer are subject to the regulation of each country.
Adherence to the basic rules of radiation safety should provide adequate protection.

- Do not eat, drink, smoke or apply cosmetics where radioactive materials are used.
- Do not pipette radioactive solutions by mouth.
- Avoid direct contact with all radioactive materials by using protective articles such as lab
  coats and disposable gloves.
- All radiological work should be done in a designated area.
- Radioactive materials should be stored in original containers in a designated area.
- Laboratory equipment and glassware which are subject to contamination should be segregated to prevent cross-contamination of different radio-isotopes.
- Any radioactive spills should be taken care of immediately in accordance with established procedures.
- All radioactive materials must be disposed of in accordance with the prevailing regulations and guidelines of the agencies jurisdiction over the laboratory.

For more information, see Material Safety Data sheet (MSDS).

XVI. BIBLIOGRAPHY

1. S. Oparil, E. Haber, N. Engl. J.
   Med. 291, 389-401, 446-457 (1974).

2. W.F. Ganong.
   Review of Medical Physiology, 6th ed., 342-344 (1973).
   Lange Medical Publications, Los Althos, CA.

3. J. Voigt, B. Wittmann-Liebold, H. Köster.
   Eur. J. Biochem, 122, 183-191 (1982).

4. O. Ganten, J.L. Minnich, P. Granger, K.Hayduk, H.M. Brecht, A. Barbeau, R. Boucher, J. Genest, Science 173, 64-65 (1971).

5. A. Leaf, G.W. Liddle, in
   "Textbook of Endocrinology" p. 938 ed. R.H. Williams,
   W.B. Saunders Co., Philadelphia (1974).

6. A. Saye.
   Hypertension, 216-221 (1983).

7. V.J. Dzan.
   Circulation 77 (Suppl 1.1) (1988).

8. Ch. Klett.
   Clin. Exp. Hypert. 9, 2027-2047 (1987).

9. J.F.E. Mann.
   Clin. Exp. Hypert. 10, 151-168 (1988).

10. J.J. Morton.
    D.J. Webb, Clinical Science 68, 482-484 (1985).

XVII. SUMMARY OF THE PROTOCOL

	Total count	NSB	Calibrator (0)	Calibrator s (1-6)	Control s	Sample s
Assay buffer	-	600µL	400 µL	-	-	-
Calibrators	-	-	-	400 µL	-	-
Controls	-	-	-		400 µL	-
Samples	-	-	-		-	400 µL
Antiserum	-	-	200µL	200µL	200µL	200µL
Vortex and incubate for 6 hours at 2-8°C						
^{125}I Tracer	200 µL					
Vortex and incubate for 18-22 hours at 2-8°C						
Double Ab Solid phase	-	100 µL				
Vortex and incubate for 30-60 min at 2-8°C						
Vortex and centrifuge for 15 min (1700 g) at 4°C						
Decant or aspirate the supernatant and count the radioactivity of the residue						

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

DIAsource Catalogue Nr : RB320RUO	Revision nr : 200701

Revision date : 01/07/2020