



Renin Activity LIA

For the quantitative determination of plasma renin activity in EDTA plasma

For Research Use Only. Not For Use In Diagnostic Procedures.

Catalog Number: 11-RENHU-L02

Size: 2 x 96 wells

Version: 6.0 - ALPCO 1.0

Intended Use

The Renin Activity LIA is for the quantitative determination of Plasma Renin Activity (PRA) in human plasma by a chemiluminescent immunoassay. This kit is for research use only. Not for use in diagnostic procedures.

Principle of the Assay

This kit measures plasma renin activity and the results are expressed in terms of mass of angiotensin-I (Ang-I) generated per volume of human plasma per unit time (ng/mL.h).

The blood sample is collected in a tube that contains EDTA. The plasma is separated and either stored frozen or kept at room temperature for immediate use. Samples should not be chilled on ice or stored at temperatures between 0 and 10°C during collection or processing before adjustment of pH, as this could lead to overestimation of renin activity. Before the start of the immunoassay, a protease inhibitor and the Generation Buffer is added to the plasma sample, which will prevent Angiotensin-I (Ang-I) in plasma from degrading. The pH of the plasma sample should be around 6.0 after the addition of the supplied Generation Buffer. The plasma sample is split in two and the fractions are incubated at 0-4°C (in ice bath) and 37°C, respectively for 90 minutes or longer, to allow the generation of Ang-I by plasma renin at 37°C. Optionally, the pH can be adjusted to 6.5 or 7.4. Adjustment of pH is a critical step during the assay, acidification of plasma to pH 3.3 or lower for prolonged time with subsequent return to neutral pH causes irreversible activation of the renin (Derkx et al., 1987), on the other side incubation at pH higher than 8.0 can destroy renin. During the immunoassay incubation, another set of protease inhibitors are involved, which function to stop the new generation as well as degradation of Ang-I to smaller peptides.

The immunoassay of Ang-I is a competitive assay that uses two incubations, with a total assay incubation time of less than two hours. During the first incubation, unlabeled Ang-I (present in the standards, controls and plasma samples) competes with biotinylated Ang-I to bind to the anti-Ang-I antibody. In the second incubation, the labelled Streptavidin-HRP conjugate, binds to the immobilized Ang-I-Biotin. The washing and decanting procedures remove unbound materials. The luminescent HRP substrate is added and the light generated (RLU) is measured in a microplate reader. The RLU values are inversely proportional to the concentration of Ang-I in the sample. A set of calibrators is used to plot a standard curve from which the concentrations of Ang-I in the samples can be directly read.

Research Applications

Measurement of PRA is important for researching hypertension. In particular, determination of plasma renin activity can help in the study of primary hyperaldosteronism (5-13% of hypertensive cases). Research indicates that PRA, in contrast to the determination of renin concentration, is a more accurate indicator of primary hyperaldosteronism (PHA), because of several reasons:

1. PRA is the expression of the rate of Ang-I formation through the enzymatic action of renin on its substrate, angiotensinogen, therefore PRA depends not only on renin concentration but also on the concentration of angiotensinogen which is ignored in the renin concentration assay.

2. Plasma renin concentration assay does not ensure sensitivity in low renin states, while the sensitivity of the PRA assay can be enhanced by increasing the incubation time during the generation step (Sealey et al., 2005).
3. When an inhibitor is bound to the renin active site PRA is inhibited, whereas the presence of the inhibitor does not affect the recognition of renin by currently available immunoassays, therefore total renin concentration does not always correlate with plasma renin activity (Campbell et al., 2009).

Renin liberates angiotensin-I from angiotensinogen. Angiotensin-I is transformed to angiotensin-II largely in pulmonary circulation by angiotensin converting enzyme (ACE). Angiotensin-II raises blood pressure by direct arteriolar vasoconstriction, promoting sodium retention, and stimulating the secretion of aldosterone from the adrenal cortex. Aldosterone also exerts an effect to restore sodium balance and lift arterial pressure. Accurate measurement of the concentration of circulating angiotensin-II is challenging because of its instability in blood samples. Aldosterone concentration can be easily determined using the Aldosterone Immunoassay Kit (11-ALDHU-E01-PLAS).

Procedural Cautions and Warnings

1. Users should have a thorough understanding of this protocol for the successful use of this kit. Reliable performance will only be attained by strict and careful adherence to the instructions provided.
2. Ang-I is presently not included in any external QC schemes. Therefore, each laboratory is suggested to establish its own internal QC materials and procedure for assessing the reliability of results.
3. When the use of water is specified for dilution or reconstitution, use deionized or distilled water.
4. All kit reagents and samples should be at room temperature and mixed gently but thoroughly before use. Avoid repeated freezing and thawing of reagents and plasma samples.
5. A calibrator curve must be established for every run. The kit controls should be included in every run and fall within established confidence limits.
6. Do not mix various lot numbers of kit components within a test and do not use any component beyond the expiration date printed on the label.
7. The luminescent substrate solutions and the prepared working substrate solution are sensitive to light and should always be stored in dark bottles away from direct sunlight.
8. To prevent contamination of reagents, use a new disposable pipette tip for dispensing each reagent, sample, standard and controls.
9. Improper procedural techniques, imprecise pipetting, incomplete washing as well as improper reagent storage may be indicated when assay values for the controls do not reflect established ranges. The performance of this assay is markedly influenced by the correct execution of the washing procedure.

Limitations

1. This kit is specifically designed and validated for the determination of renin activity/Ang-I generation in EDTA plasma. Other sources of material should be validated before being tested.

2. The Ang-I level depends on multiple factors, including renin activity, renin substrate concentration, the plasma pH, temperature and selection of inhibitors. Therefore, only carefully prepared plasma samples are suitable for this assay.
3. Bacterial contamination, repeated freeze and thaw cycles and dilution of plasma samples may affect the assay result.
4. The interpretation of the results should recognize the conditions that can affect renin secretion, such as sodium and potassium intake, posture, medications like diuretics, chlonidine, beta-blockers, estroproggestogens and peripheral vasodilators.
5. Do not use grossly hemolyzed, lipemic, or icteric plasma, or any sample that was not handled properly according to the sample collection instructions.
6. This kit is for research use only. It should not be used for diagnostic procedures. Please note: the occurrence of heterophilic antibodies in individuals regularly exposed to animals or animal products has the potential of causing interference in immunological tests.

Safety: Cautions and Warnings

Potentially Biohazardous Material

All reagents in this kit should be considered potential biohazards and handled with the same precautions as applied to any blood sample. Human plasma samples should be handled as if capable of transmitting infections and in accordance with good laboratory practices.

Chemical Hazards

Avoid contact with reagents containing PMSF and hydrogen peroxide. If contact occurs with these or any other reagents in this kit, wash with plenty of water.

Reagents and Equipment Needed But Not Provided

1. Disodium EDTA (2 mg/mL blood) collection tubes
2. Single and multi-channel pipettes and disposable tips
3. Distilled or deionized water
4. Disposable test tubes (glass or polypropylene)
5. Plate shaker capable of 200 rpm
6. Microplate luminescence reader
7. 37°C incubator
8. Ice bath
9. 95% Ethanol
10. Centrifuge
11. Vortex
12. Automatic strip washer (recommended)
13. Timer

REAGENTS PROVIDED

1. Generation Buffer

Contents: Buffer and non-toxic antibiotic.
Volume: 5 mL/bottle
Storage: Refrigerate at 2-8°C
Stability: 12 months or as indicated on label.

2. PMSF – Requires Preparation

Contents: One bottle containing phenylmethylsulfonyl fluoride (PMSF).

Storage: Refrigerate at 2-8°C

Stability: 12 months or as indicated on label.

Preparation: Reconstitute by adding 0.5 mL of 95% ethanol to the bottle and vortex for two minutes to completely dissolve the PMSF. Refrigerate after first use, vortex again to re-dissolve contents. Do not keep the bottle open unnecessarily.

3. Rabbit Anti-Ang-I Antibody Coated Microwell Plate

Contents: Two 96 well pre-coated microwell plates in a resealable pouch with desiccant.

Storage: Refrigerate at 2-8°C

Stability: 12 months or as indicated on label.

4. Angiotensin-I-Biotin Conjugate

Contents: One bottle containing buffer, protease inhibitors, Angiotensin-I-Biotin conjugate and a non-mercury preservative.

Volume: 30 mL/bottle

Storage: Refrigerate at 2-8°C

Stability: 12 months in unopened vial or as indicated on label.

5. Streptavidin-Horseradish Peroxidase Conjugate Concentrate – Requires Preparation X100

Contents: Streptavidin-HRP conjugate in a protein-based buffer with a non-mercury preservative.

Volume: 0.5 mL/vial

Storage: Refrigerate at 2-8°C

Stability: 12 months in an unopened vial or as indicated on label.

Preparation: Dilute the conjugate concentrate 1:100 in assay buffer before use. The working conjugate solution is stable for 8 hours; discard the unused solution after this period.

6. Angiotensin-I Calibrators

Contents: Eight vials containing synthetic angiotensin-I peptide in a protein-based buffer with a non-mercury preservative. The calibrators are calibrated against the World Health Organization reference reagent NIBSC code 86/536.

Calibrator concentrations*: 0, 0.2, 0.5, 1.5, 4, 10, 25, 60 ng/mL.

* Approximate value - please refer to vial labels for exact lot specific concentrations.

Volume: Calibrator A: 2 mL/vial
Calibrators B-H: 0.7 mL/vial

Storage: Refrigerate at 2-8°C

Stability: 12 months in unopened vials or as indicated on label.

7. Controls

Contents: Two vials containing angiotensin-I in a protein-based buffer with a non-mercury preservative. Refer to vial labels for acceptable range.
Volume: 0.7 mL/vial
Storage: Refrigerate at 2-8°C
Stability: 12 months in unopened vials or as indicated on label.

8. Assay Buffer

Contents: One bottle containing protein-based buffer with a non-mercury preservative.
Volume: 40 mL/bottle
Storage: Refrigerate at 2-8°C
Stability: 12 months or as indicated on label.

9. Wash Buffer Concentrate – Requires Preparation x10

Contents: Two bottles containing buffer with a non-ionic detergent and a non-mercury preservative.
Volume: 50 mL/bottle
Storage: Refrigerate at 2-8°C
Stability: 12 months or as indicated on label.
Preparation: Dilute 1:10 in distilled or deionized water before use. If one whole plate is to be used dilute 50 mL of the wash buffer concentrate in 450 mL of water.

10. LIA Substrate Reagent A – Requires Preparation

Contents: One vial containing luminol plus enhancer.
Volume: 2 mL/vial
Storage: Refrigerate at 2-8°C
Stability: 12 months or as indicated on label.
Preparation: See preparation of LIA working substrate solution.

11. LIA Substrate Reagent B – Requires Preparation

Contents: One vial containing stabilized peroxide solution.
Volume: 4 mL/vial
Storage: Refrigerate at 2-8°C
Stability: 12 months or as indicated on label.
Preparation: See preparation of LIA working substrate solution.

12. LIA Substrate Reagent C – Requires Preparation

Contents: One bottle of buffer with a non-mercury preservative.
Volume: 40 mL/bottle
Storage: Refrigerate at 2-8°C
Stability: 12 months or as indicated on label.
Preparation: See preparation of LIA working substrate solution.

Preparation of LIA Working Substrate Solution

Preparation: In a clean dry container, mix 1 part of reagent A with 2 parts of reagent B in 20 parts of reagent C. This yields the ready to use substrate solution.

If the whole plate is to be used prepare working substrate solution as follows: combine 0.9 mL of reagent A with 1.8 mL of reagent B and 18 mL of reagent C; this yields 20.7 mL working substrate.

It is suggested to wait at least 2 minutes after mixing, prior to using the working substrate.

Stability: Working substrate solution is stable for 8 hours at room temperature; discard the unused solution after this period.

Sample Collection and Storage

A minimum of 0.5 mL of plasma is required per duplicate determination. Appropriate sample collection is essential to the accurate determination of angiotensin-I. The *in-vitro* generation and degradation of angiotensin-I can be minimized by the following recommended collection procedure:

1. Collect 2 mL of blood into an EDTA venipuncture tube or syringe.
2. Centrifuge blood for 15 minutes at 5000 rpm at room temperature.
3. Transfer plasma sample to a test tube at room temperature.
4. If samples are to be assayed immediately, proceed to the Angiotensin-I generation procedure; otherwise, freeze samples immediately at -20°C or less. Avoid freezing and thawing samples more than once.

Angiotensin-I Generation Procedure

1. If a freshly drawn plasma sample is being used proceed to step 2. If frozen plasma samples are being used, thaw them as follows: quickly bring frozen plasma samples to room temperature by placing the tubes in a container of room temperature water.
2. Transfer 0.5 mL of the plasma sample into a test tube.
3. Add 5 µL of the PMSF solution to the 0.5 mL of plasma sample (1:100 ratio). Vortex the tube to mix thoroughly.
4. Add 50 µL of the Generation Buffer to the treated sample from step 3 (1:10 ratio). Vortex the tube again to mix thoroughly.
5. Divide the treated sample from step 4 equally into two aliquots by transferring 0.25 mL into two test tubes. Incubate one aliquot for 90 minutes or longer (do not exceed 180 minutes) at 37°C. Place the second aliquot on an ice bath (0°C). Be sure to record the incubation time used for the aliquots (needed for calculations).
6. At the end of the incubation period place the 37°C aliquot on the ice bath for 5 minutes to cool it down quickly.
7. Bring both aliquots to room temperature by placing in a bath with room temperature water for 5-10 minutes (do not exceed 10 minutes).

Assay Procedure

1. Allow all kit components to reach room temperature. Remove the required number of microwell strips and assemble into the plate frame.
2. Pipette 50 μL of each calibrator, control, and treated plasma sample (both 37°C and 0°C aliquots) into correspondingly labelled wells, in duplicate.
3. Pipette 100 μL of the angiotensin-I-biotin conjugate into each well (the use of a multichannel pipette is recommended).
4. Incubate on a plate shaker (~200 rpm) for 60 minutes at room temperature.
5. Wash the wells 5 times, each time with 300 μL per well of diluted wash buffer. After washing, tap the plate firmly against absorbent paper to remove any residual liquid (the use of an automatic strip washer is recommended). The performance of this assay is markedly influenced by the correct execution of the washing procedure.
6. Pipette 150 μL of the streptavidin-HRP conjugate working solution into each well (the use of a multichannel pipette is recommended).
7. Incubate on a plate shaker (~200 rpm) for 30 minutes at room temperature.
8. Wash the wells 3 times, each time with 300 μL per well of diluted wash buffer (the use of an automatic strip washer is recommended).
9. Rinse the wells 3 times, each time with 300 μL per/well of distilled or deionized water. After rinsing, tap the plate firmly against absorbent paper to remove any residual liquid (the use of an automatic strip washer is recommended).
10. Pipette 150 μL of the LIA substrate working solution into each well (the use of a multichannel pipette is recommended).
11. Measure the relative light units (RLUs) in each well on a microwell plate luminometer between 10-30 minutes after addition of the substrate. It is recommended to set the measuring time to 1 second per well.

Calculations

1. Using immunoassay software, choose either a 4-parameter or 5-parameter curve fitting method for calculating results.
2. If a sample reads more than the 60ng/mL then dilute it with calibrator A at a dilution of no more than 1:10 and rerun the sample. The result obtained should be multiplied by the dilution factor.
3. Calculate the plasma renin activity (PRA) in each sample using the following equation:

$$PRA = \left\{ \frac{[\text{Ang-I (37°C)}] - [\text{Ang-I (0°C)}]}{\text{Time (hrs)}} \times \right\} 1.11$$

Where time (hrs) is the incubation time used during the generation step.

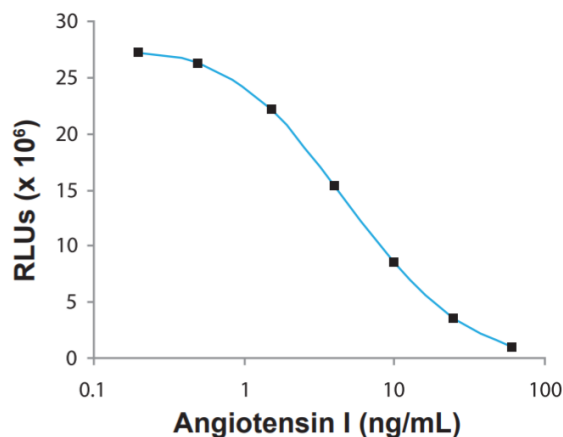
Typical Tabulated Data

Sample data only. Do not use to calculate results.

Calibrator	Ang-I (ng/mL)	Mean RLU	%RLU/RLU ₀
A	0	28600560	100
B	0.2	27197120	95.1
C	0.5	26326945	92.1
D	1.5	22172900	77.5
E	4	15361535	53.7
F	10	8511020	29.8
G	25	3617255	12.6
H	60	1073600	3.8

Typical Calibration Curve

Sample curve only, do not use to calculate results.



Performance Characteristics

Analytical Sensitivity

The limit of detection (LoD) was determined from the analysis of 40 samples of the blank and a low value sample and it was calculated as follows:

$$LoD = \mu_B + 1.645\tilde{\sigma}_B + 1.645\tilde{\sigma}_s$$

Where $\tilde{\sigma}_B$ and $\tilde{\sigma}_s$ are the standard deviation of the blank and low value sample and μ_B is the mean value of the blank.

$$LoD = 0.22\text{ng/mL of Angiotensin I}$$

Analytical Specificity (Cross-Reactivity)

The following compounds were tested for cross-reactivity using the Abraham method with angiotensin-I cross reacting at 100%:

Antigen	Sequence	% Cross-Reactivity
Angiotensin-I	DRVYIHPFHL	100
Angiotensin 1-9	DRVYIHPFH	0.012
Angiotensin-II	DRVYIHPF	<0.01
Angiotensin-III	RVYIHPF	<0.01
Angiotensin 1-5	DRVYI	<0.01
Renin Substrate human	DRVYIHPFHLVIHN	<0.01

Recovery

Spiked samples were prepared by adding defined amounts of angiotensin-I to three plasma samples. The results (in ng/mL) are tabulated below:

Sample	Observed Result	Expected Result	Recovery %
1.Unspiked	1.38	-	-
+0.48	1.88	1.86	101
+1.92	3.11	3.3	94
+5.77	6.87	7.15	96
+11.53	11.72	12.92	91
2.Unspiked	2.07	-	-
+0.48	2.56	2.55	100
+1.92	3.96	3.99	99
+5.77	7.56	7.84	96
+11.53	12.88	13.61	95
3.Unspiked	2.9	-	-
+0.48	3.4	3.38	101
+1.92	4.75	4.82	99
+5.77	8.89	8.67	103
+11.53	13.67	14.44	95

Linearity

Three serum samples were diluted with calibrator A. The results (in ng/mL) are tabulated below:

Sample	Obs. Result	Exp. Result	Recovery %
1	7.83	-	-
1:2	3.91	3.92	100
1:4	1.99	1.96	102
1:8	0.99	0.98	101
1:16	0.48	0.49	98
2	8.82	-	-
1:2	4.86	4.41	110
1:4	2.30	2.21	104
1:8	1.12	1.10	102
1:16	0.53	0.55	96
3	11.83	-	-
1:2	5.77	5.92	98
1:4	2.62	2.96	89
1:8	1.30	1.48	88
1:16	0.66	0.74	89

Interference

Interference testing was performed according to CLSI guideline EP7-A2. Plasma samples with varying levels of angiotensin-I were spiked with potential interfering substances at recommended levels and analyzed. Results were compared to the same plasma samples with no extra substances added to calculate the % interference.

$$\text{Interference (\%)} = \frac{[\text{Ang I (Spiked sample)}] - [\text{Ang I (Native Sample)}]}{[\text{Ang I (Native Sample)}]} \times 100$$

Interferent	Added Interferent Concentration	% Interference
Hemoglobin	1 g/ L	-5.6
	2 g/L	-10
Bilirubin Unconjugated	20 µM (12 mg/L)	+3.2
	500 µM (300 mg/L)	-1.6
Bilirubin Conjugated*	20 µM (16 mg/L)	-2.8
	500 µM (400 mg/L)	+7.2
Hemoglobin + Bilirubin	1 g/L + 20 µM	-5.5
	1 g/L + 500 µM	-8.7
	2 g/L + 20 µM	-15.2
	2 g/L + 500 µM	-15.9

Triglycerides (2C-10C)	3.7 mM	+0.4
	37 mM	+17
Triglycerides (8C-16C)	3.7 mM	+9.8
	37 mM	+11
HSA	40 g/L	-10
	60 g/L	-16
Biotin	50 µg/mL	-6.8
	10 µg/mL	-9.4

*Taurobilirubin

Intra-Assay Precision

Four samples were assayed ten times each on the same calibrator curve. The results (in ng/mL) are tabulated below:

Sample	Mean	SD	CV%
1	0.447	0.041	9.07
2	2.563	0.161	6.27
3	7.007	0.458	6.54
4	11.81	0.769	6.51

Inter-Assay Precision

Four samples were assayed in ten different tests. The results (in ng/mL) are tabulated below:

Sample	Mean	SD	CV%
1	1.79	0.15	8.54
2	5.32	0.49	9.22
3	8.86	0.66	7.49
4	14.37	1.40	9.72

Comparative Studies

A Plasma Renin Activity ELISA kit (x) was compared with the Renin Activity LIA kit (y). The comparison of 12 plasma samples yielded the following linear regression results:

$$y = 0.97x - 0.05$$

$$r = 0.96$$

References

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