Renin Activity ELISA

For the quantitative determination of Plasma Renin Activity (PRA) in human plasma

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

Catalog Number: 11-RENHU-E02
Size: 2 x 96 Wells
Version: 6.0 – ALPCO- November 12, 2018

INTENDED USE

For the quantitative determination of Plasma Renin Activity (PRA) in human plasma by an enzyme immunoassay. For Research Use Only. Not for Use in Diagnostic Procedures.

PRINCIPLE OF THE TEST

This kit measures PRA and the results are expressed in terms of mass of angiotensin-I (Ang-I) generated per volume of human plasma in 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 are added to the plasma sample, which will prevent Angiotensin-I (Ang-I) in plasma from degradation. The pH of the plasma sample should be around 6.0 after the addition of the supplied generation buffer. The plasma sample is split into two aliquots and the fractions are incubated at 0-4°C (in ice bath) and 37ºC, 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), while incubation at a 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, unlabelled 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 colorimetric HRP substrate is added and, after stopping the color development reaction, the light absorbance (OD) is measured in a microwell plate reader. The absorbance 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 and controls can be directly read.

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 substrate (TMB) solution is 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 applied.
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 test.
3. Bacterial contamination, repeated freeze and thaw cycles and dilution of plasma samples may affect the assay result.
4. Do not use grossly hemolyzed, lipemic, or icteric plasma, or any sample that was not handled properly according to the instructions.

SAFETY CAUTIONS AND WARNINGS

POTENTIAL BIOHAZARDOUS MATERIAL

All reagents in this kit should be considered a potential biohazard 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, TMB, hydrogen peroxide and sulfuric acid. If contact with any of these or other reagents in this kit occurs, 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 disposal tips
3. Distilled or deionized water
4. Disposable test tubes (glass or polypropylene)
5. Plate shaker
6. Microwell plate absorbance reader equipped with a 450 nm filter
7. 37˚C incubator
8. Ice bath
9. 95% Ethanol
10. Timer
11. Centrifuge
12. Vortex

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, and vortex again to re-dissolve contents after storing. 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 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. TMB Substrate
    Contents: One bottle containing tetramethylbenzidine and hydrogen peroxide in a non-DMF or DMSO containing buffer
    Volume: 32 mL/vial
    Storage: Refrigerate at 2-8°C
    Stability: 12 months or as indicated on label

11. Stop Solution
    Contents: One bottle containing 1M sulfuric acid.
    Volume: 12 mL/bottle
    Storage: Refrigerate at 2-8°C
    Stability: 12 months or as indicated on label

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 now 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 with 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 in an ice bath (0°C). Be sure to record the incubation time used for the aliquots as this is used for calculations.
6. At the end of the incubation period place the 37 °C aliquot in 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 microplate 5 times with 300 µL of diluted wash buffer per well. After washing, tap the plate firmly against absorbent paper to remove any residual liquid (the use of an automatic strip washer is strongly 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 5 times with the same procedure as in step 5.
9. Pipette 150 µL of the TMB substrate into each well (the use of a multichannel pipette is recommended). Incubate on a plate shaker (~200 rpm) for 10 to 15 minutes at room temperature.
10. Add 50 µL of stop solution to each well and mix thoroughly by gently tapping the plate.
11. Measure the absorbance at 450 nm in all wells with a microplate reader between 0-20 minutes after addition of the stop solution.

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 60 ng/mL, dilute the sample 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.

   Note: Samples must be diluted only after they have undergone the angiotensin-I generation procedure; do not dilute any samples before performing the angiotensin-I generation procedure.
3. Calculate the plasma renin activity (PRA) in each sample using the following equation:

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

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

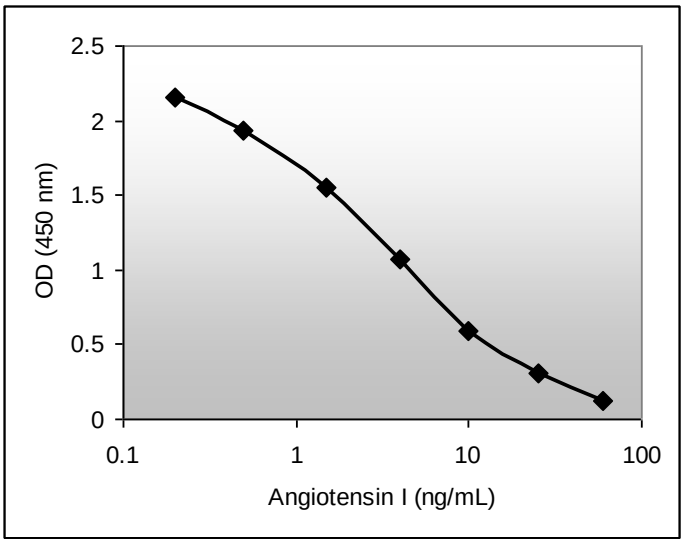
TYPICAL TABULATED DATA

Sample data only. Do not use to calculate results.

Calibrator	Mean OD (450 nm)	Ang-I (ng/mL)
A	2.303	0
B	2.156	0.2
C	1.937	0.5
D	1.552	1.5
E	1.066	4
F	0.591	10
G	0.311	25
H	0.122	60

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 = µB + 1.645σB + 1.645σS,

where σB and σS are the standard deviation of the blank and low value sample and µB is the mean value of the blank.

LoD = 0.14 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	0.015
Angiotensin-II	DRVYIHPF	<0.001
Angiotensin-III	RVYIHPF	<0.001
Angiotensin 1-5	DRVYI	<0.001
Renin Substrate human	DRVYIHPFHLVIHN	0.001

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	0.86	-	-
+0.48	1.43	1.34	107
+1.92	2.82	2.78	101
+5.77	6.47	6.63	98
+11.53	10.58	12.40	85
2.Unspiked	2.84	-	-
+0.48	3.30	3.32	99
+1.92	5.34	4.77	112
+5.77	8.84	8.61	103
+11.53	13.08	14.38	91
3.Unspiked	9.45	-	-
+0.48	9.92	9.93	100
+1.92	11.35	11.37	100
+5.77	13.82	15.22	91
+11.53	17.62	20.99	84

LINEARITY

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

Sample	Obs.Result	Exp.Result	Recovery %
1	10.96	-	-
1:2	5.739	5.48	105
1:4	2.718	2.74	99
1:8	1.423	1.37	104
1:16	0.776	0.685	113
2	15.798	-	-
1:2	8.273	7.899	105
1:4	3.934	3.950	100
1:8	1.948	1.975	99
1:16	1.146	0.987	116
3	30.7	-	-
1:2	16.142	15.350	105
1:4	7.477	7.675	97
1:8	3.542	3.838	92
1:16	1.574	1.919	82

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.

$$\textit{Interference}\ (\%) = \frac{[\textbf{Ang I(Spiked sample)}] - [\textbf{AngI(Native sample)}]}{[\textbf{AngI(Native sample)}]}\ \textbf{x 100}$$

Interferent	Added Interferent Concentration	% Interference
Hemoglobin	1 g/ L	-3.0
	2 g/L	-3.8
Bilirubin Unconjugated	20 µM (12 mg/L)	0
	500 µM (300 mg/L)	0
Bilirubin Conjugated*	20 µM (16 mg/L)	+3.0
	500 µM (400 mg/L)	+13.5
Hemoglobin + Bilirubin	1 g/L + 20 µM	-0.4
	1 g/L + 500 µM	-0.1
	2 g/L + 20 µM	-3.9
	2 g/L + 500 µM	-12.4
Triglycerides (2C-10C)	3.7 mM	+4.8
	37 mM	+16.9
Triglycerides (8C-16C)	3.7 mM	-0.6
	37 mM	+2.2
HSA	40 g/L	-2.2
	60 g/L	-9.6

*Taurobilirubin

INTRA-ASSAY PRECISION

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

Sample	Mean	SD	CV%
1	2.3	0.2	8.7
2	3.6	0.2	6.8
3	7.0	0.4	6.3
4	13.3	0.9	7.0

INTER-ASSAY PRECISION

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

Sample	Mean	SD	CV%
1	0.48	0.03	7.12
2	0.82	0.04	5.32
3	9.46	0.45	4.81
4	11.70	0.64	5.44

COMPARATIVE STUDIES

The PRA ELISA kit (y) was compared with a competitor's PRA RIA kit (x). The comparison of 73 plasma samples yielded the following linear regression results:

y = 0.93x - 0.08, r = 0.97

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