Diagnostics Biochem Canada

Plasma Renin Activity (PRA) ELISA

REF CAN-RA-4600

ASSAY PROCEDURE

- Bring kit components to room temperature. Prepare working solutions.
- Pipette 50 µL of each calibrator, control and pre-treated specimen sample.
- Pipette 100 µL of Biotin Conjugate into each well.
- Incubate on a microplate shaker for 60 minutes at room temperature.
- Wash 5 times.
- Pipette 150 µL of Streptavidin-HRP Conjugate Working Solution into each well.
- Incubate on a microplate shaker for 30 minutes at room temperature.
- Wash 5 times. Pipette 150 µL of TMB Substrate.
- Incubate on a plate shaker for 10–15 minutes at room temperature.
- Pipette 50 µL of Stopping Solution.
- Read the plate on a microplate reader at 450 nm.

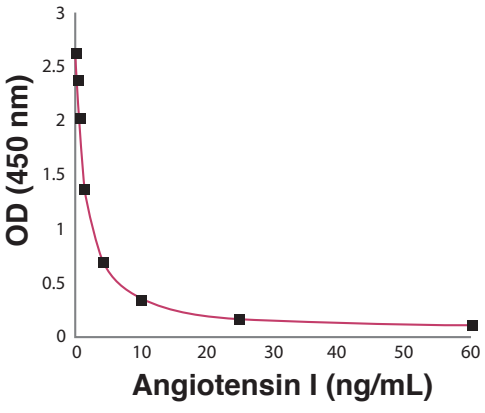
Measurement of PRA is important for the clinical evaluation of hypertensive patients. In particular, determination of plasma renin activity can help in the diagnosis of primary hyperaldosteronism (5–13% of hypertensive cases) and assist in the therapy and management of other forms of hypertension.

PRINCIPLE OF THE TEST

Prior to testing plasma samples with the PRA ELISA, a specimen pre-treatment step is required. First, a protease inhibitor (PMSF) is added to the sample to prevent the degradation of angiotensin-I. Next, the generation buffer is added to bring the pH of the sample to approximately 6.0. The plasma sample is then pipetted into two aliquots. One aliquot is incubated at 0°C (ice bath) and the other is incubated at 37°C. Angiotensin-I will be generated by plasma renin in the fraction incubated at 37°C.

The PRA ELISA is a competitive immunoassay. In the first incubation step, competition occurs between angiotensin-I present in calibrators, controls, specimen samples and an angiotensin-I-biotin conjugate (biotin conjugate) for a limited number of anti-angiotensin-I antibody binding sites on the microplate wells. During this incubation, protease inhibitors are present to prevent the degradation of angiotensin-I into smaller peptides.

In the second incubation step, streptavidin-HRP conjugate is added, which binds specifically to any bound biotin conjugate. Unbound streptavidin HRP conjugate is removed by a washing step. Next, the TMB substrate (enzyme substrate) is added which reacts with HRP to form a blue coloured product that is inversely proportional to the amount of angiotensin-I present. The enzymatic reaction is terminated by the addition of the stopping solution, converting the blue colour to a yellow colour. The absorbance is measured on a microplate reader at 450 nm. A set of calibrators is used to plot a calibrator curve from which the concentration of angiotensin-I in specimen samples and controls can be directly read.



The plasma renin activity concentration in the plasma sample is calculated from the angiotensin-I concentration in the 0°C and 37°C aliquots and the generation time used. The plasma renin activity results are expressed in terms of the mass of angiotensin-I generated per volume of human plasma per unit of time (ng/mL/h).

PERFORMANCE CHARACTERISTICS

SENSITIVITY

The analytical sensitivity study was performed according to the CLSI EP17-A2 guideline. The Limit of Background (LoB), Limit of Detection (LoD) and Limit of Quantitation (LoQ) for both Angiotensin-I and PRA are summarized in the table below:

Parameter	Angiotensin-I (ng/mL)	PRA (ng/mL/h)
LoB	0.093	0.024
LoD	0.166	0.059
LoQ	0.166	0.090

SPECIFICITY (CROSS-REACTIVITY)

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

Compound	% Cross-Reactivity
Angiotensin I	100
Angiotensin II	< 0.001
Angiotensin III	< 0.001
Angiotensin 1-5	< 0.001
Angiotensin 1-7	< 0.001
Angiotensin 1-9	0.0122
Renin Substrate	0.015

INTERFERENCES

An interference study was performed according to the CLSI EP07-Ed3 guideline. Three human plasma samples were spiked with potentially interfering substances. No significant interference was detected up to the concentrations shown in the table below.

Interferent	Test Concentration
Acetaminophen	30 µg/mL
Acetylcysteine	15 mg/dL
Acetylsalicylic Acid	3 mg/dL
Ampicillin Na	7.5 mg/dL
Bilirubin Conjugated	20 mg/dL
Bilirubin Unconjugated	40 mg/dL
Biotin	2.4 µg/mL
Captopril	1000 ng/mL
Captopril disulfide	10 µg/mL
Cathepsin B	100 ng/mL
Cathepsin D	10 ng/mL
Cefoxitin Na	300 mg/dL
Cyclosporine	0.18 mg/dL
Doxycycline HCl	1.8 mg/dL
Enalaprilat dihydrate	200 ng/mL

Interferent	Test Concentration
Furosemide (Lasix)	50 µg/mL
Haemoglobin	1.25 g/L
HAMA	1000 ng/mL
Heparin	3300 U/L
Human Serum Albumin	52 g/L
Ibuprofen	21.9 mg/dL
Insulin	150 µIU/mL
Levodopa	0.75 mg/dL
Methyldopa	2.25 mg/dL
Metronidazole	12.3 mg/dL
Nicardipine (Loxen)	200 ng/mL
Phenylbutazone	32.1 mg/dL
Rheumatoid Factor (RF)	200 IU/mL
Rifampicin	4.8 mg/dL
Theophylline	25 µg/mL

RECOVERY

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

Sample	Observed Result	Expected Result	Recovery %
1 Unspiked	1.09	-	-
+ 1	2.16	2.09	103.3
+ 15	15.3	16.1	95.0
+ 50	41.8	51.1	81.8
2 Unspiked	1.72	-	-
+ 1	2.70	2.72	99.3
+ 15	16.8	16.7	100.6
+ 50	54.1	51.7	104.6
3 Unspiked	1.01	-	-
+ 1	1.76	2.01	87.6
+ 15	12.7	16.0	79.4
+ 50	41.7	51.0	81.8

LINEARITY

The linearity study was according to the CLSI EP06-Ed2 guideline using three human EDTA plasma samples.

Each plasma sample was pre-treated according to the Angiotensin-I Generation Procedure to produce a 0°C and 37°C aliquot. Each aliquot was diluted using calibrator A at several equidistant concentration levels and up to a 1:10 dilution. Samples were tested in quadruplicate, and the results compared to the predicted concentrations. The statistical analysis shows that the assay is sufficiently linear up to a 1:10 dilution when using calibrator A as the diluent. The results (in ng/mL/h) are tabulated below:

Sample	Observed Result	Expected Result	Recovery %
1	33.7	-	-
1:2	17.9	16.9	105.9
1:4	8.33	8.43	98.8
1:10	3.24	3.37	96.1
2	7.11	-	-
1:2	3.33	3.56	93.5
1:4	1.59	1.78	89.3
1:10	0.60	0.71	84.5
3	1.66	-	-
1:2	0.68	0.83	81.9
1:4	0.33	0.42	78.6
1:10	0.13	0.17	76.5

REFERENCE RANGES

As for all clinical assays each laboratory should collect data and establish their own range of expected normal values. Data from Literature reference.[1]

N	PRA Mean (ng/mL.h)	PRA Range (10^{th}-90^{th} percentile) (ng/mL.h)
533	0.75	0.06–4.69

1. Brossaud J, Corcuff JB. Pre-Analytical and Analytical Considerations for the Determination of Plasma Renin Activity. *Clin Chim Acta*. 2009; 410(1–2):90–2.

Ordering Information: **REF CAN-RA-4600**

DIAGNOSTICS BIOCHEM CANADA
384 NEPTUNE CRESCENT • LONDON, ONTARIO, CANADA • N6M 1A1
TEL: 519-681-8731 • FAX: 519-681-8734 • dbc@dbc-labs.com • www.dbc-labs.com