

ENG

Product Data Sheet:

PLASMA RENIN ACTIVITY ELISA

Catalogue number:
RCD032R

For research use only!

B|G| BioVendor
R&D®

BioVendor – Laboratorní medicína a.s.

Karásek 1767/1, 621 00 Brno, Czech Republic

+420 549 124 185

info@biovendor.com

sales@biovendor.com

www.biovendor.com

1. INTENDED USE	3
2. PRINCIPLE OF THE TEST	3
3. CLINICAL APPLICATIONS	4
4. PROCEDURAL CAUTIONS AND WARNINGS	4
5. LIMITATIONS	5
6. SAFETY CAUTIONS AND WARNINGS	5
7. REAGENTS AND EQUIPMENT NEEDED BUT NOT PROVIDED	6
8. REAGENTS PROVIDED	6
9. SPECIMEN COLLECTION AND STORAGE	8
10. ANGIOTENSIN-I GENERATION PROCEDURE	9
11. ASSAY PROCEDURE	9
12. CALCULATIONS	10
13. TYPICAL TABULATED DATA	10
14. TYPICAL CALIBRATOR CURVE	11
15. PERFORMANCE CHARACTERISTICS	11
16. COMPARATIVE STUDIES	14
17. EXPECTED NORMAL VALUES	14
18. REFERENCE	15

1. INTENDED USE

For the quantitative determination of Plasma Renin Activity (PRA) in human plasma by an enzyme immunoassay.

For research use only.

2. 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, this could lead to overestimation of renin activity. Before the start of immunoassay a protease inhibitor and the Generation buffer is 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 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 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 microplate 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.

3. CLINICAL APPLICATIONS

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. 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 BioVendor immunoassay kit (RCD030R).

4. PROCEDURAL CAUTIONS AND WARNINGS

- 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.
- 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.
- When the use of water is specified for dilution or reconstitution, use deionized or distilled water.
- All kit reagents and specimens should be at room temperature and mixed gently but thoroughly before use. Avoid repeated freezing and thawing of reagents and plasma specimens.
- A calibrator curve must be established for every run. The kit controls should be included in every run and fall within established confidence limits.
- 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.
- The substrate (TMB) solution is sensitive to light and should always be stored in dark bottles away from direct sunlight.
- To prevent contamination of reagents, use a new disposable pipette tip for dispensing each reagent, sample, standard and controls.
- 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!

5. LIMITATIONS

- 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.
- 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.
- Bacterial contaminations, repeated freeze and thaw cycles and dilution of plasma samples may affect the assay result.
- The interpretation of the results should recognise the conditions that can affect renin secretion, such as sodium and potassium intake, posture, medications like diuretics, chlonidine, beta-blockers, estroprogestogens and peripheral vasodilators.
- Do not use grossly haemolysed, lipaemic, icteric plasma, and any sample that was not handled properly according to the instruction.
- The results obtained with this kit should not be used as the sole basis for clinical diagnosis. For example, the occurrence of heterophilic antibodies in patients regularly exposed to animals or animal products has the potential of causing interferences in immunological tests. Consequently, the clinical diagnosis should include all aspects of a patient's background including the frequency of exposure to animal products if false results are suspected.

6. SAFETY CAUTIONS AND WARNINGS

6.1 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 specimen. Human plasma samples should be handled as if capable of transmitting infections and in accordance with good laboratory practices.

6.2. CHEMICAL HAZARDS

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

7. REAGENTS AND EQUIPMENT NEEDED BUT NOT PROVIDED

- Disodium EDTA (2 mg/mL blood) collection tubes
- Single and multi-channel pipettes and disposal tips
- Distilled or deionized water
- Disposable test tubes (glass or polypropylene)
- Plate shaker
- Microplate absorbance reader equipped with a 450 nm filter
- 37°C incubator
- Ice bath
- 95% Ethanol

8. 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 Microplate

Contents: Two 96-well pre-coated microplates 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 (HRP) 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 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 nonmercury 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 proteinbased 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 as indicated on label.

11. Stopping Solution

Contents: One bottle containing 1M sulfuric acid.

Volume: 12 mL/bottle

Storage: Refrigerate at 2–8°C

Stability: 12 months as indicated on label.

9. SPECIMEN 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.

10. 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
2. temperature by placing the tubes in a container with room temperature water.
3. Transfer 0.5 mL of the plasma sample into a test tube.
4. Add 5 μ L of the PMSF solution to the 0.5 mL of plasma sample (1:100 ratio). Vortex the tube to mix thoroughly.
5. Add 50 μ L of the generation buffer to the treated sample from step 3 (1:10 ratio). Vortex the tube again to mix thoroughly.
6. 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 as this is used for calculations.
7. At the end of the incubation period place the 37°C aliquot on the ice-bath for 5 minutes to cool it down quickly.
8. Bring both aliquots to room temperature by placing in a bath with room temperature water for 5–10 minutes (do not exceed 10 minutes).

11. ASSAY PROCEDURE

1. Allow all kit components to reach room temperature.
2. Remove the required number of well strips and assemble into the plate frame.
3. Pipette 50 μ L of each calibrator, control and treated plasma sample (both 37°C and 0°C aliquots) into correspondingly labelled wells in duplicate.
4. Pipette 100 μ L of the angiotensin-I-biotin conjugate into each well (the use of a multichannel pipette is recommended).
5. Incubate on a plate shaker (~200 rpm) for 60 minutes at room temperature.
6. Wash the wells 5 times each time with 300 μ L/well of diluted wash buffer. After washing tap the plate firmly against absorbent paper to remove any residual
7. 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!
8. Pipette 150 μ L of the streptavidin-HRP conjugate working solution into each well (the use of a multichannel pipette is recommended).
9. Incubate on a plate shaker (~200 rpm) for 30 minutes at room temperature.
10. Wash the wells 5 times with the same procedure as in step 5.
11. Pipette 150 μ L 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.
12. Add 50 μ L of stopping solution to each well and mix thoroughly by gently tapping the plate.
13. Measure the absorbance at 450 nm in all wells with a microplate reader between 0–20 minutes after addition of the stopping solution.

12. CALCULATIONS

- Using immunoassay software, choose either a 4-parameter or 5-parameter curve fitting method for calculating results.
- If a sample reads more than 60 ng/mL then dilute the sample (that has undergone the angiotensin-I generation procedure) 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.
- Calculate the plasma renin activity (PRA) in each sample using the following equation:

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

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

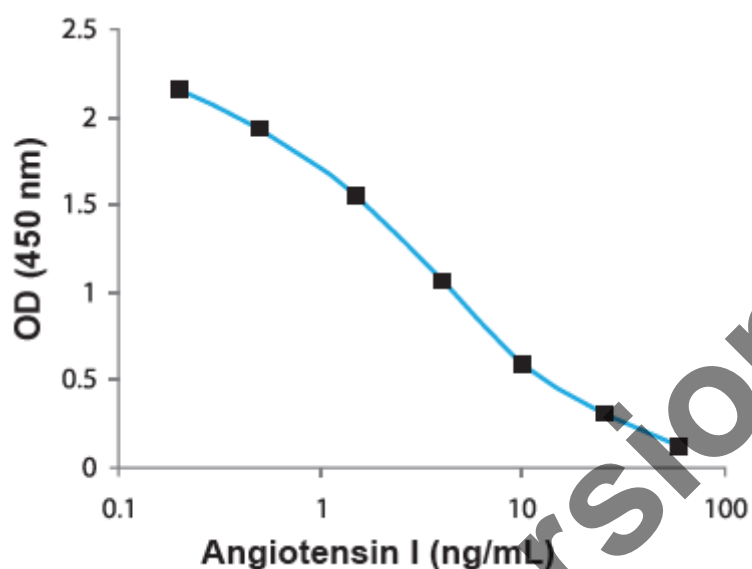
13. TYPICAL TABULATED DATA

Sample data only. Do not use to calculate results.

Calibrator	Mean OD (450 nm)	Ang-I (ng/ml)
A	2.803	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

14. TYPICAL CALIBRATOR CURVE

Sample curve only. Do not use to calculate results.



15. PERFORMANCE CHARACTERISTICS

15.1 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:

$$\text{LoD} = \mu_B + 1.645\sigma_B + 1.645\sigma_S,$$

where σ_B and σ_S are the standard deviation of the blank and low value sample and μ_B is the mean value of the blank.

LoD = 0.14 ng/mL of Angiotensin I

15.2 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

15.3. Recovery

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

Sample	Obs Result	Exp. 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

15.4. Linearity

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

Serum	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

15.5. 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.

Interferent	Added Interferent Concentration	% Interference
Haemoglobin	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
Haemoglobin + 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

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

15.6 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

15.7 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

16. COMPARATIVE STUDIES

The BioVendor Plasma Active Renin 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:

$$y = 0.93x - 0.08, r = 0.97$$

17. EXPECTED NORMAL VALUES

As for all clinical assays each laboratory should collect data and establish their own range of expected normal values. Data presented here were from samples incubated at pH 6.0 during the generation step (Brossaud and Corcuff, 2009).

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

18. REFERENCE

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.
2. Bystrom CE, et al. Plasma Renin Activity by LC-MS/MS:
3. Development of a Prototypical Clinical Assay Reveals a Subpopulation of Human Plasma Samples With Substantial Peptidase Activity. *Clin Chem*. 2010; 56(10):1561–9.
4. Campbell DJ, et al. Activity Assays and Immunoassays for Plasma Renin and Prorenin: Information Provided and Precautions Necessary for Accurate Measurement. *Clin Chem*. 2009; 55(5):867–77.
6. Cartledge S, Lawson N. Aldosterone and Renin Measurements. *Ann Clin Biochem*. 2000; 37(Pt 3):262–78.
7. Derkx FH, et al. Two-step Prorenin-Renin Conversion. Isolation of an Intermediary Form of Activated Prorenin. *J Biol Chem*. 1987; 262(6):2472–7.
8. Hartman D, et al. Direct Renin Assay and Plasma Renin Activity Assay Compared. *Clin Chem*. 2004; 50(11):2159–61.
9. Pimenta E, Calhoun D. Response to “Effective” Plasma Renin Activity: A Derived Measure for Assessing Residual Plasma Renin Activity in Patients Taking Angiotensin- Converting Enzyme Inhibitors or Angiotensin Receptor Blockers. *Hypertension*. 2010; 55:e17.
10. Reudelhuber TL. Prorenin, Renin, and Their Receptor: Moving Targets. *Hypertension*. 2010; 55(5):1071–4.
11. Sealey JE, et al. Plasma Renin Methodology: Inadequate Sensitivity and Accuracy of Direct Renin Assay for Clinical Applications Compared With the Traditional Enzymatic Plasma Renin Activity Assay. *J Hypertens*. 1995; 13(1):27–30.
12. Sealey JE. Plasma Renin Activity and Plasma Prorenin Assays. *Clin Chem*. 1991; 37(10 Pt 2):1811–9.
13. Sealey JE, et al. Plasma Renin and Aldosterone Measurements in Low Renin Hypertensive States. *Trends Endocrinol Metab*. 2005; 16(3):86–91.
14. Ulmer PS, Meikle AW. Sample Requirements for Plasma Renin Activity and Immunoreactive Renin. *Clin Chem*. 2000; 46(9):1442–4.



BioVendor – Laboratorní medicína a.s.
Karásek 1767/1, 621 00 Brno, Czech Republic
+420 549 124 185
info@biovendor.com
sales@biovendor.com
www.biovendor.com

Example Version

