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Instructions for use

Plasma Renin Activity (PRA) ELISA

REF **MS E-5600R**

2°C–8°C

Σ 96

RUO For research use only – Not for use in diagnostic procedures

Plasma Renin Activity (PRA) ELISA

1. INTENDED PURPOSE & USE

For the quantitative measurement Plasma Renin Activity (PRA) in human EDTA plasma by an ELISA (Enzyme-Linked Immunosorbent Assay).

This kit is intended for professional use only and is for laboratory use only. For *research* use only. Intended to be used manually but may be adaptable to open automated analyzers. The user is responsible for validating the performance of this kit with any automated analyzers.

2. LIMITATIONS RELATED TO INTENDED PURPOSE & USE

1. This test is not intended to be used for screening purposes.
2. This test is not intended for home testing or self-testing.
3. The kit is calibrated for the determination of renin activity in human plasma. The kit is not calibrated for the determination of renin activity in other specimens of human or animal origin.
4. Although common interfering substances have been evaluated with this test, other substances that have not been evaluated such as drugs and the occurrence of heterophilic antibodies in individuals regularly exposed to animals or animal products have the potential of causing interferences.
5. The angiotensin-I level depends on multiple factors, including renin activity, renin substrate concentration, 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.
6. The interpretation of the results should recognize that some conditions can affect renin secretion, such as sodium and potassium intake, posture, medications like diuretics, clonidine, beta-blockers, and peripheral vasodilators.

3. SUPPLEMENTAL INFORMATION

PRA is based on renin releasing angiotensin-I from angiotensinogen. Angiotensin-I is transformed to angiotensin-II largely in pulmonary circulation by the angiotensin-converting enzyme (ACE).

PRA and renin concentration assays provide different information about plasma renin. First, PRA is the expression of the rate of angiotensin-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 overlooked in the renin concentration assays. Second, plasma renin concentration assays do 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. Third, PRA is influenced by inhibitors, whereas the presence of inhibitors does not affect the recognition of renin by currently available immunoassays, therefore total renin concentration does not always correlate with plasma renin activity.

4. 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 standards, 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 standards is used to plot a standard 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).

5. PROCEDURAL CAUTIONS AND WARNINGS

1. This kit is for use by trained laboratory personnel (professional use only). For laboratory research use only.
2. Practice good laboratory practices when handling kit reagents and specimens. This includes:
   - Do not pipette by mouth.
   - Do not smoke, drink, or eat in areas where specimens or kit reagents are handled.
   - Wear protective clothing and disposable gloves.
   - Wash hands thoroughly after performing the test.
   - Avoid contact with eyes; use safety glasses; in case of contact with eyes, flush eyes with water immediately and contact a doctor.
3. 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.
4. Do not use the kit beyond the expiry date stated on the label.
5. If the kit reagents are visibly damaged, do not use the test kit.
6. Do not use kit components from different kit lots within a test and do not use any component beyond the expiration date printed on the label.
7. All kit reagents and specimens must be brought to room temperature and mixed gently but thoroughly before use. Avoid repeated freezing and thawing of specimens.
8. When the use of water is specified for dilution or reconstitution, use deionized or distilled water.
9. Immediately after use, each individual component of the kit must be returned to the recommended storage temperature stated on the label.
10. A standard curve must be established for every run.
11. It is recommended to all customers to prepare their own control materials or plasma pools which should be included in every run at a high and low level for assessing the reliability of results.
12. The controls (included in kit) must be included in every run and their results must fall within the ranges stated in the quality control certificate; a failed control result might indicate improper procedural techniques or pipetting, incomplete washing, or improper reagent storage.
13. When dispensing the substrate and stopping solutions, do not use pipettes in which these liquids will come into contact with any metal parts.
14. The TMB Substrate is sensitive to light and should remain colourless if properly stored. Instability or contamination may be indicated by the development of a blue colour, in which case it should not be used.
15. Do not use grossly hemolyzed, grossly lipemic, icteric or improperly stored plasma.
16. Collected specimen samples must be immediately processed (centrifuged) and the plasma must be either stored frozen or kept at room temperature for immediate use. Samples should not be chilled on ice or stored at temperatures between 0 – 10 °C during collection or processing as this could lead to overestimation of renin activity.
17. Samples or controls containing azide or thimerosal are not compatible with this kit, they may lead to false results.
18. If sample values are above the angiotensin-I measuring range of the ELISA kit, they may be further diluted and retested. Only standard A may be used to dilute plasma samples. The use of any other reagent may lead to false results. Samples must be diluted only after they have undergone the angiotensin-I generation procedure.
19. Avoid microbial contamination of reagents.
20. To prevent the contamination of reagents, use a new disposable pipette tip for dispensing each reagent, sample, standard, and control.
21. To prevent the contamination of reagents, do not pour reagents back into the original containers.
22. Kit reagents must be regarded as hazardous waste and disposed of according to local and/or national regulations.
23. Consumables used with the kit that are potentially biohazardous (e.g., pipette tips, bottles or containers containing human materials) must be handled according to biosafety practices to minimize the risk of infection and disposed of according to local and/or national regulations relating to biohazardous waste.
24. This kit contains 1 M sulfuric acid in the stopping solution component. Do not combine acid with waste material containing sodium azide or sodium hypochlorite.
25. The use of safety glasses, and disposable plastic, is strongly recommended when manipulating biohazardous or bio-contaminated solutions.
26. Proper calibration of the equipment used with the test, such as the pipettes and absorbance microplate reader, is required.
27. If a microplate shaker is required for the assay procedure, the type and speed of shaker required is stated in the REAGENTS AND EQUIPMENT NEEDED BUT NOT PROVIDED section. Both the type and speed of shaker used can influence the optical densities and test results. If a different type of shaker and/or speed is used, the user is responsible for validating the performance of the kit.
28. Do not reuse the microplate wells, they are for SINGLE USE only.
29. To avoid condensation within the microplate wells in humid environments, do not open the pouch containing the microplate until it has reached room temperature.

6. SAFETY CAUTIONS AND WARNINGS

6.1 BIOHAZARDS

The reagents should be considered a potential biohazard and handled with the same precautions applied to blood specimens. All human specimens should be considered a potential biohazard and handled as if capable of transmitting infections and in accordance with good laboratory practices.

6.2 CHEMICAL HAZARDS

Avoid direct contact with any of the kit reagents. Specifically avoid contact with the TMB Substrate (contains tetramethylbenzidine), Stopping Solution (contains sulfuric acid) and PMSF. If contacted with any of these reagents, wash with plenty of water and refer to SDS for additional information.

7. SPECIMEN COLLECTION, STORAGE AND PRE-TREATMENT

7.1 Specimen Collection & Storage

A minimum of 0.5 ml of EDTA plasma is required per duplicate determination. Proper sample collection is essential for the accurate determination of angiotensin-I. The *research* generation and degradation of angiotensin-I can be minimized by following the recommended collection and processing procedure as stated below.

1. Collect at least 2 ml of venous blood into an appropriately labelled EDTA blood collection tube.
2. Centrifuge the sample at room temperature for 15 minutes at 2000 g.
3. Transfer the plasma sample into a new labelled storage tube.
4. If samples are to be assayed immediately, proceed to the Specimen Pre-Treatment section, otherwise store at room temperature for up to 6 hours or freeze at -20 °C or lower for up to 30 days. Avoid more than two freeze-thaw cycles.

Consider all human specimens as possible biohazardous materials and take appropriate precautions when handling.

7.2 Specimen Pre-Treatment & Storage

Prior to being tested, all processed plasma specimens must be pre-treated according to the Angiotensin-I generation procedure as stated below. At the end of this procedure, there will be two pre-treated aliquots per plasma sample, a 0 °C aliquot and a 37 °C aliquot.

Angiotensin-I Generation Procedure

1. If a freshly processed plasma sample is being used, proceed to step 2. If a frozen plasma sample is being used, thaw the sample quickly by placing the tube in a room temperature water bath.
2. Pipette 0.5 ml of the plasma sample into a new sample tube.
3. Pipette 5 µl of the PMSF solution (see section *9. Reagents Provided*, *7. PMSF*, for preparation instructions) into the tube containing the plasma sample from step 2 (1:100 ratio). Vortex the tube to mix thoroughly.
4. Pipette 50 µl of the generation buffer into the tube containing the treated sample from step 3 (1:10 ratio). Vortex the tube to mix thoroughly.
5. Pipette 0.25 ml of the sample from step 4 into a new sample tube. There will now be two aliquots of the treated plasma sample. Label one as **0 °C** and the other as **37 °C**.
6. Simultaneously place the 37 °C labelled tube into a 37 °C incubator and place the 0 °C labelled tube into an ice bath (0 – 4 °C) for 90 minutes or longer (do not exceed 180 minutes). Be sure to record the incubation time used, as this is required to calculate the plasma renin activity.
7. At the end of the incubation period place the 37 °C tube in the ice bath for 5 minutes to cool it down quickly.
8. If the generated samples will be tested immediately, bring both sample tubes (0 °C and 37 °C) to room temperature by placing them in a water bath with room temperature water for 5 – 10 minutes. The samples are now ready for testing.
9. If the generated samples will be tested at a later time, immediately freeze both sample tubes (0 °C and 37 °C) at -20 °C or lower for up to 3 months. Prior to use, bring the frozen generated samples to room temperature by placing them in a water bath with room temperature water for 5 – 10 minutes. The samples are now ready for testing.

⚠ **Do not** pre-treat the standards and kit controls; they are provided in a ready to use format.

8. REAGENTS AND EQUIPMENT NEEDED BUT NOT PROVIDED

1. Calibrated single-channel pipette to dispense 5 µl, 50 µl, 250 µl and 500 µl.
2. Calibrated multi-channel pipettes to dispense 50 µl, 100 µl and 150 µl.
3. Calibrated multi-channel pipettes to dispense 300 µl (if washing manually).
4. Automatic microplate washer (recommended).
5. Microplate shaker:
   a. Orbital shaker (3 mm diameter) set to 600 rpm or
   b. Reciprocating shaker (1.5" stroke length) set to 180 oscillations/minute.
6. Disposable pipette tips.
7. Distilled or deionized water.
8. Calibrated absorbance microplate reader with a 450 nm filter and an upper OD limit of 3.0 or greater.
9. Polypropylene tubes for sample processing and pre-treatment (e.g., polypropylene microcentrifuge tubes).
10. A 37 °C incubator.
11. A 0 – 4 °C ice bath.
12. Ethanol (94% or higher concentration).
13. Water bath.

9. REAGENTS PROVIDED

1. MS E-5631 96 **Microplate –** Ready to Use

Contents:	Two anti-angiotensin-I polyclonal antibody-coated 96-well (12x8) microplates, each in a resealable pouch with desiccant.
Storage:	2 – 8 °C
Stability:	Unopened: Stable until the expiry date printed on the label. After Opening: Stable for ten weeks.

2. MS E-5610 BIOTIN-AB **Biotin Conjugate –** Ready to Use

Contents:	One bottle containing Angiotensin-I-Biotin conjugate in a protein-based buffer with protease inhibitors and a non-mercury preservative.
Volume:	30 ml/bottle
Storage:	2 – 8 °C
Stability:	Unopened: Stable until the expiry date printed on the label. After Opening: Stable for ten weeks.

3. MS E-5640 CONJUGATE-CONC 100x **Streptavidin-HRP Conjugate Concentrate –** Concentrated; Requires Preparation

Contents:	One bottle containing Streptavidin-Horse Radish Peroxidase (HRP) conjugate in a protein-based buffer with a non-mercury preservative.
Volume:	0.5 ml/bottle
Storage:	2 – 8 °C
Stability:	Unopened: Stable until the expiry date printed on the label. After Opening: Stable for ten weeks. Following Preparation: The HRP conjugate working solution is stable for 8 hours at room temperature following preparation.
Preparation of Streptavidin-HRP Conjugate Working Solution:	**X100** **Dilute 1:100 Before Use** Dilute 1:100 in assay buffer before use (e.g., 20 µl of conjugate concentrate in 1.98 ml of assay buffer). If one whole microplate is to be used, dilute 200 µl of conjugate concentrate in 19.8 ml of assay buffer. Discard any that is left over.

4. **Standards** and **Controls** – Ready to Use

Cat. no.	Symbol	Standard	Concentration*	Volume/bottle
MS E-5601	STANDARD A	Standard A	0 ng/ml	2.0 ml
MS E-5602	STANDARD B	Standard B	0.2 ng/ml	1.0 ml
MS E-5603	STANDARD C	Standard C	0.5 ng/ml	1.0 ml
MS E-5604	STANDARD D	Standard D	1.5 ng/ml	1.0 ml
MS E-5605	STANDARD E	Standard E	4 ng/ml	1.0 ml
MS E-5606	STANDARD F	Standard F	10 ng/ml	1.0 ml
MS E-5607	STANDARD G	Standard G	25 ng/ml	1.0 ml
MS E-5608	STANDARD H	Standard H	60 ng/ml	1.0 ml
MS E-5651	CONTROL 1	Control 1	Refer to the QC certificate for the target values and acceptable ranges.	1.0 ml
MS E-5652	CONTROL 2	Control 2		1.0 ml

* Listed below are approximate concentrations, please refer to vial labels for exact concentrations.

Contents: Eight bottles of standard containing specified angiotensin-I concentrations. Protein-based buffer with a non-mercury preservative. Prepared by spiking buffer with defined quantities of angiotensin-I.
The standards are calibrated against the World Health Organization reference reagent NIBSC code 86/536.
Two bottles of control containing different angiotensin-I concentrations. Protein-based buffer with a non-mercury preservative. Prepared by spiking buffer with defined quantities of angiotensin-I.

Storage: 2 – 8 °C

Stability: Unopened: Stable until the expiry date printed on the label.
After Opening: Stable for ten weeks.

5. **MS E-5613** ASSAY-BUFF **Assay Buffer –** Ready to Use

Contents: One bottle containing a protein-based buffer with a non-mercury preservative.

Volume: 40 ml/bottle

Storage: 2 – 8 °C

Stability: Unopened: Stable until the expiry date printed on the label.
After Opening: Stable for ten weeks.

6. **MS E-5616** BUFF **Generation Buffer –** Ready to Use

Contents: One bottle containing a buffer and a non-toxic antibiotic.

Volume: 5 ml/bottle

Storage: 2 – 8 °C

Stability: Unopened: Stable until the expiry date printed on the label.
After Opening: Stable for ten weeks.

Hazards identification:

H332 Harmful if inhaled.
H317 May cause an allergic skin reaction.
H334 May cause allergy or asthma symtoms or breathing difficulties if inhaled.

7. **MS E-5614** PMSF **Phenylmethylsulfonyl fluoride –** Requires Preparation

Contents: Two tubes containing phenylmethylsulfonyl fluoride (PMSF).

Quantity: 2 x tubes

Storage: 2 – 8 °C

Stability: Unopened: Stable until the expiry date printed on the label.
Following Preparation: Stable for 2 months at 2 – 8 °C.

Preparation of PMSF Working Solution: Reconstitute by adding 0.5 ml of ethanol (94% or higher concentration) to the tube. Cap the tube and vortex for two minutes to completely dissolve. Refrigerate after first use, vortex again to re-dissolve contents before use. Do not keep the bottle open unnecessarily.

Hazards identification:

H301 Toxic if swallowed.
H314 Causes severe skin burns and eye damage.
H318 Causes serious eye damage.

8. MS E-5655 SUBSTRATE **TMB Substrate –** Ready to Use

Contents: One bottle containing tetramethylbenzidine and hydrogen peroxide in a non-DMF or DMSO containing buffer.

Volume: 32 ml/bottle

Storage: 2 – 8 °C

Stability: Unopened: Stable until the expiry date printed on the label.
After Opening: Stable for ten weeks.

9. MS E-5680 STOP-SOLN **Stopping Solution –** Ready to Use

Contents: One bottle containing 1 M sulfuric acid.

Volume: 12 ml/bottle

Storage: 2 – 8 °C

Stability: Unopened: Stable until the expiry date printed on the label.
After Opening: Stable for ten weeks.

Hazards identification:

H315 Causes skin irritation.
H319 Causes serious eye irritation.

10. AA E-0030 WASH-CONC 10x **Wash Buffer Concentrate –** Concentrated; Requires Preaparation

Contents: Two bottles containing buffer with a non-ionic detergent and a non-mercury preservative.

Volume: 50 ml/bottle (Quantity: 2 bottles)

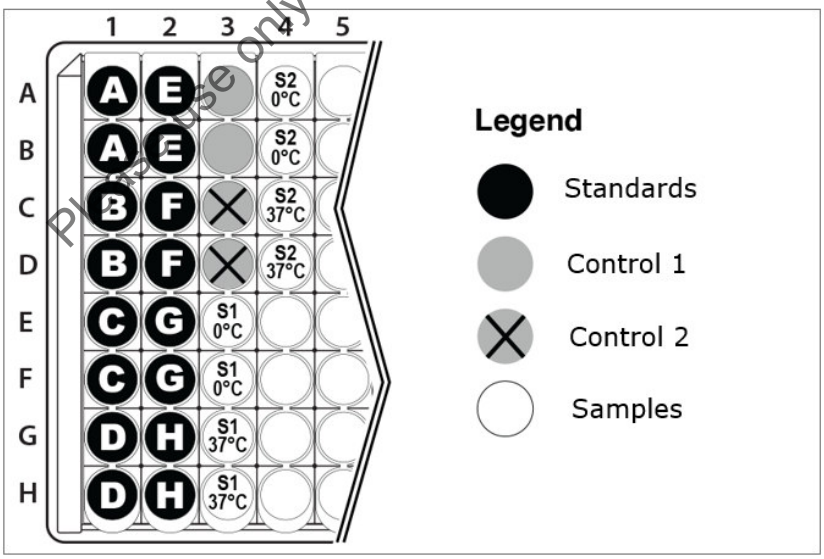
Storage: 2 – 8 °C

Stability: Unopened: Stable until the expiry date printed on the label.
After Opening: Stable for ten weeks.
Following Preparation: The wash buffer working solution is stable for 2 weeks following preparation, assuming Good Laboratory Practices are adhered to. To prevent microbial growth, prepare the wash buffer working solution in a clean container and store under refrigerated conditions (2 – 8 °C) when not in use.

Preparation of Wash Buffer Working Solution: **X10** **Dilute 1:10 Before Use**

Dilute 1:10 in distilled or deionized water before use. If one whole microplate is to be used, dilute 50 ml of the wash buffer concentrate in 450 ml of distilled or deionized water.

10. RECOMMENDED ASSAY LAYOUT



11. ASSAY PROCEDURE

Specimen Pre-Treatment:	
⚠	**All specimens that will be tested must be pre-treated before being tested (see section *7.2. Specimen Pre-Treatment & Storage*). Do not pre-treat the standards and kit controls as they are provided ready to use.**
All kit components, controls and specimen samples must reach room temperature prior to use. Standards, controls, and specimen samples should be assayed in duplicate. Once the procedure has been started, all steps should be completed without interruption.	
1.	After all kit components have reached room temperature, **mix** gently by inversion.
2.	**Prepare** the Streptavidin-HRP Conjugate Working Solution and Wash Buffer Working Solution (See section *9. Reagents Provided* section*, 3. Streptavidin-HRP Conjugate Concentrate* and *11. Wash Buffer Concentrate*).
3.	**Prepare** all specimen samples that will be tested. Refer to section *7.2. Specimen Pre-Treatment & Storage*. For each plasma sample, both the 0 °C and 37 °C pre-treated samples must be run together within the same test.
4.	**Plan** the microplate wells to be used for standards, controls, and samples. See section *10. Recommended Assay Layout.* Remove the strips from the microplate frame that will not be used and place them in the bag with desiccant. Reseal the bag with the unused strips and return it to the refrigerator.
5.	**Pipette 50 µl** of each standard, control, and pre-treated specimen sample (both 0 °C and 37 °C aliquots) into assigned wells.
6.	**Pipette 100 µl** of the Biotin Conjugate into each well (the use of a multi-channel pipette is recommended).
7.	**Incubate** the microplate on a microplate shaker** for **60 minutes** at room temperature.
8.	Wash the microplate wells with an automatic microplate washer (preferred) or manually as stated below. Automatic: Using an automatic microplate washer, perform a **5-cycle** wash using **300 µl/well** of Wash Buffer Working Solution (5 x 300 µl). One cycle consists of aspirating all wells then filling each well with 300 µl of Wash Buffer Working Solution. After the final wash cycle, aspirate all wells and then tap the microplate firmly against absorbent paper to remove any residual liquid. Manually: For manual washing, perform a **5-cycle** wash using **300 µl/well** of Wash Buffer Working Solution (5 x 300 µl). One cycle consists of aspirating all wells by briskly emptying the contents of the wells over a waster container, then pipetting 300 µl of Wash Buffer Working Solution into each well using a multi-channel pipette. After the final wash cycle, aspirate all wells by briskly emptying the contents over a waste container and then tap the microplate firmly against absorbent paper to remove any residual liquid.
9.	**Pipette 150 µl** of the Streptavidin-HRP Conjugate Working Solution into each well (the use of a multi-channel pipette is recommended).
10.	**Incubate** the microplate on a microplate shaker** for **30 minutes** at room temperature.
11.	**Wash** the microplate wells again as stated in step 8.
12.	**Pipette 150 µl** of TMB Substrate into each well (the use of a multi-channel pipette is recommended).
13.	**Incubate** the microplate on a microplate shaker** for **15 minutes** at room temperature.
14.	**Pipette 50 µl** of Stopping Solution into each well (the use of a multi-channel pipette is recommended) in the same order and speed as was used for addition of the TMB Substrate. Gently tap the microplate frame to mix the contents of the wells.
15.	**Measure** the optical density (absorbance) in the microplate wells using an absorbance microplate reader set to 450 nm, within 20 minutes after addition of the Stopping Solution.

** See section *8. Reagents And Equipment Needed But Not Provided* for microplate shaker options.

12. CALCULATIONS

1. Calculate the mean optical density for each standard, control and specimen sample duplicate.
2. Use a 4-parameter or 5-parameter curve fit with immunoassay software to generate a standard curve.
3. The immunoassay software will calculate the concentrations of the controls and specimen samples using the mean optical density values and the standard curve.
4. Using the obtained concentrations of Angiotensin-I (Ang-I) in the 37 °C and 0 °C aliquots and the generation time used, calculate the plasma renin activity (PRA) in each sample using the following equation:

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

5. If a sample reads more than 60 ng/ml then dilute the sample (that has undergone the angiotensin-I generation procedure) with standard A at a dilution of no more than 1:10 and rerun the sample. The result obtained must 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.

13. QUALITY CONTROL

When assessing the validity of the test results, the following criteria should be evaluated:

1. The standard A mean optical density meets the acceptable range as stated in the QC Certificate.
2. The standard with the highest concentration meets the % binding acceptable range as stated in the QC Certificate. % Binding = (OD of standard/OD of standard A) x 100.
3. The values obtained for the kit controls are within the acceptable ranges as stated in the QC certificate.
4. The results of any external controls that were used meet the acceptable ranges.

14. TYPICAL DATA

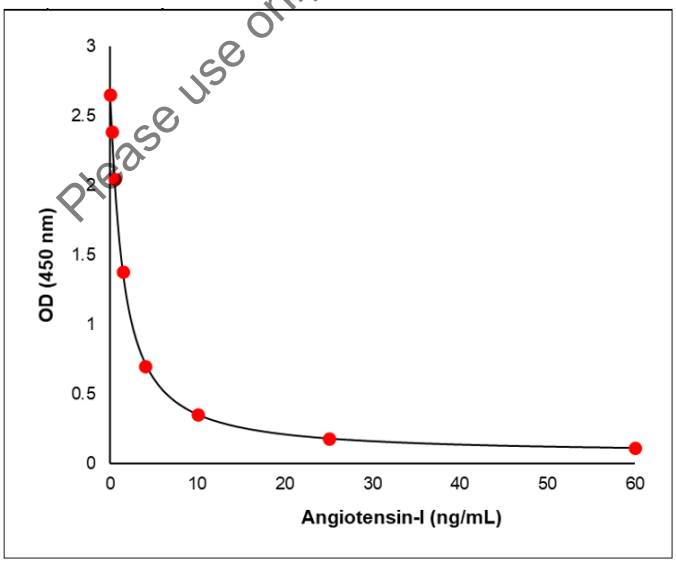
14.1 TYPICAL TABULATED DATA

Sample data only. **Do not** use to calculate results.

Standard	Mean OD (450 nm)	% Binding	Angiotensin-I (ng/ml)
A	2.654	100	0
B	2.388	90	0.2
C	2.044	77	0.5
D	1.383	52	1.5
E	0.701	26	4
F	0.353	13	10
G	0.182	7	25
H	0.114	4	60
Unknown	1.634	–	1.0

14.2 TYPICAL STANDARD CURVE

Sample curve only. **Do not** use to calculate results.



15. PERFORMANCE CHARACTERISTICS

15.1 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

15.2 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	0.122%
Renin Substrate	0.015%

15.3 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
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
Triglycerides	1000 mg/dl

15.4 PRECISION

The precision studies were performed according to the CLSI EP05-A3 guideline.

Repeatability

Five EDTA plasma samples covering the range of the assay (from 0.17 to 52.1 ng/ml of angiotensin-I) were run by one operator with one kit lot over 20 days, two runs per day and two replicate measurements per run, producing a total of 80 measurements per sample (20x2x2 design for each sample). The position of the samples in the microplate was randomly changed from one day to another. The PRA generation times spanned 90 – 180 minutes. Data were analyzed with a two-way nested ANOVA and are summarized in the table below

Sample	Mean, PRA, ng/ml/hr	Repeatability		Within Laboratory	
		SD, PRA, ng/ml/hr	CV	SD, PRA, ng/ml/hr	CV
1	0.322	0.0367	11.4%	0.0615	19.1%
2	4.568	0.2060	4.5%	0.3565	7.8%
3	1.672	0.0768	4.6%	0.1275	7.6%
4	7.924	0.5005	6.3%	0.8042	10.1%
5	22.886	1.7496	7.6%	3.0223	13.2%

Reproducibility

Reproducibility was evaluated across three locations using an experimental design model 3x5x5 (3 locations x 5 testing days x 5 replicates per sample per day). The study included five plasma samples covering the kit assay range and the two kit controls. The generation time changed from day to day covering the full range suggested in this IFU (90 – 180 minutes). The position of the kit controls and samples in the microplate was randomized from one day to another. Data was analyzed with a two-way nested ANOVA and are summarized in the table below.

Kit Controls	Mean	Repeatability		Within Location		Reproducibility	
	ng/ml	SD, ng/ml	CV%	SD, ng/ml	CV%	SD, ng/ml	CV%
1	0.966	0.051	5.2	0.081	8.4	0.140	14.5
2	9.850	0.370	3.8	0.701	7.1	0.820	8.3
PRA	**ng/ml/h**	**SD, ng/ml/h**	**CV%**	**SD, ng/ml/h**	**CV%**	**SD, ng/ml/h**	**CV%**
S1	0.285	0.049	17.2	0.053	18.7	0.065	22.9
S2	4.416	0.247	5.6	0.596	13.5	0.596	13.5
S3	1.431	0.101	7.1	0.147	10.3	0.256	17.9
S4	7.656	0.490	6.4	0.827	10.8	0.910	11.9
S5	22.763	2.379	10.5	3.520	15.5	3.985	17.5

15.5 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 standard 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 standard 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

15.6 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

16. REFERENCE RANGES

Data from Literature reference [16].

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

17. LITERATURE

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18. CHANGE HISTORY

Previous Version:	6.0-r	**New Version:**	7.0-r
Changes:	Design change of product and new IFU format; all information in IFU was revised.		
Previous Version:	7.0-r	**New Version:**	8.0-r
	7.2 Specimen Pre-Treatment & Storage Angiotensin-I Generation Procedure 8. Added: If the generated samples will be tested immediately, …The samples are now ready for testing. 9. Added: If the generated samples will be tested at a later time, … Added information on how to bring the frozen generated samples to room temperature prior to testing. 9. Reagents Provided 9. Stopping Solution The hazard pictogram GHS05 has been replaced by the hazard pictogram GHS07.		

Symbols:

+2 +8 °C	Storage temperature		Manufacturer	Σ	Contains sufficient for <n> tests
	Use-by date	LOT	Batch code		
	Consult instructions for use	CONT	Content		
	Caution	REF	Catalogue number		Distributor
	Date of manufacture			RUO	For research use only!