EAGLE BIOSCIENCES

Plasma Renin Activity (PRA) ELISA Kit

Catalog Number:
REN31-K02 (1 x 96 wells)
For Research Use Only. Not for use in diagnostic procedures.
v. 9.0 (effective 17OCT22)

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INTENDED USE

The Eagle Biosciences Plasma Renin Activity (PRA) ELISA Kit (enzyme-linked immunoassay kit) is intended for the quantitative measurement of plasma renin activity in human EDTA plasma. The Eagle Biosciences Plasma Renin Activity (PRA) ELISA Kit is for research use only and not to be used in diagnostic procedures.

For further information about this kit, its application, or the procedures in this insert, please contact the Technical Service Team at Eagle Biosciences, Inc at www.EagleBio.com or at 866-411-8023.

PRINCIPLE OF THE ASSAY

The Eagle Biosciences Plasma Renin Activity ELISA Kit is a competitive immunoassay. 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 degradation on 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.

In the first incubation step, competition occurs between angiotensin-I present in standard, 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 from a blue colored 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 color to a yellow color. 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 of time used. The plasma renin activity results are expressed in terms of the mass angiotensin-I generated per volume of human plasma per unit of time (ng/mL/h).

LIMITATIONS RELATED TO INTENDED USE

1. This kit is intended for research use only and is not to b used for any diagnostic procedures.

PROCEDURAL WARNINGS AND PRECAUTIONS

- This kit is for use by trained laboratory personnel (professional use only). For research use only.
- 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.
- 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.
- Do not use this kit beyond the expiry date stated on the label.
- If the kit reagents are visibly damaged, do not use the test kit.

- 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.
- 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.
- When the use of water is specified for dilution or reconstitution, use deionized or distilled water.
- Immediately after use, each individual component of the kit must be returned to the recommended storage temperature stated on the label.
- A standard curve must be established for every run.
- It is recommended to all customers to prepare their own control materials or sample pools which should be included in every run at a high and low level for assessing the reliability of results.
- The controls (if applicable with this 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 reagent storage.
- When dispensing the substrate and stopping solutions, do not use pipettes in which these liquids will come into contact with any metal parts.
- The TMB Substrate is sensitive to light and should remain colorless if properly stored. Instability or contamination may be indicated by the development of a blue color, in which case it should not be used.
- Do not use grossly hemolyzed, grossly lipemic, icteric or improperly stored serum.
- Samples or controls containing azide or thimerosal are not compatible with this kit, they may lead to false results.
- Avoid microbial contamination of reagents.
- To prevent the contamination of reagents, use a new disposable pipette tip for dispensing each reagent, sample, standard, and control.
- To prevent contamination of reagents, do not pour reagents back into the original containers.
- Kit reagents must be regarded as hazardous waste and disposed of according to local and/or national regulations.
- 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.
- 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.
- The use of safety glasses, and disposable plastic, is strongly recommended when manipulating biohazardous or bio-contaminated solutions.
- Proper calibration of the equipment used with the test, such as the pipettes and absorbance microplate reader, is required.
- 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 shake and/or speed is used, the user is responsible for validating the performance of the kit.
- Do not reuse the microplate wells, they are for SINGLE USE only.
- To avoid condensation within the microplate wells in humid environments, do not open the pouch containing the microplate until it has reached room temperature.
- Any serious incident that has occurred in relation to the device shall be reported to the manufacturer and the competent authority of the Member State in which the user and/or the participant is established.
- When reading the microplate, the presence of bubbles in the wells will affect the optical densities (ODs). Carefully remove any bubbles before performing the reading step.

SAFETY CAUTIONS AND WARNINGS

BIOHAZARDS

The reagents should be considered a potential biohazard and handled with the same precautions applied to human 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.

CHEMICAL HAZARDS

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

SPECIMEN COLLECTION, STORAGE, AND PRE-TREATMENT

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 generation and degradation of angiotensin-1 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 labeled EDTA blood collection tube.
2. Centrifuge the sample at room temperature for 15 minutes at 2000g.
3. Transfer the plasma sample into a new labeled 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.

Specimen Pre-Treatment & Storage

Prior to being tested, all processed plasma specimens must be pre-treated according to the Angiotensin-1 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-1 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 Reagents provided, 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 labeled tube into a 37°C incubator and place the 0°C labeled tube into an ice bath (0-4°C) for 90 minutes 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.

REAGENTS AND EQUIPMENT NEEDED BUT NOT PROVIDED

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

REAGENTS PROVIDED

1. **Microplate**

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

2. **Biotin Conjugate**

Contents:	One bottle containing Angiotensin-I-Biotin conjugate in a protein-based buffer with protease inhibitors and a non-mercury preservative.
Format:	Ready to Use
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. **HRP Conjugate**

Contents:	One bottle containing streptavidin-HRP conjugate in a stabilizing buffer with a non-mercury preservative.
Format:	Concentrated; Requires Preparation
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:	**Dilute 1:100** in assay buffer before use (e.g., 20 uL 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. **Standard A - H**

Contents:	Eight bottles of standard containing different angiotensisn-I concentrations in a buffer with a non-mercury preservative. Prepared by spiking buffer with a defined quantity of angiotensin-I. Standards are calibrated against the World Health Organization reference reagent NIBSC code 86/536. Listed below are approximate concentrations, please refer to vial labels for exact concentrations. Concentrations: 0, 0.2, 0.5, 1.5, 4, 10, 25, 60 ng/mL
Format:	Ready to Use
Volume:	Standard A: 2.0 mL/bottle Standard B-H: 1.0 mL/bottle
Storage:	2-8°C
Stability	Unopened: Stable until the expiry date printed on the label. After Opening: Stable for ten weeks.

5. **Control 1 - 2**

Contents:	Two bottles of control containing angiotensin I in a buffer with a non-mercury preservative. Prepared by spiking buffer with defined quantities of adiponectin. Refer to the QC certificate for the target values and acceptable ranges.
Format:	Ready to Use
Volume:	1 mL/bottle
Storage:	2-8°C
Stability:	Unopened: Stable until the expiry date printed on the label. After Opening: Stable for ten weeks.

6. **Assay Buffer**

Contents:	One bottle containing a protein-based buffer with a non-mercury preservative.
Format:	Ready to Use
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.

7. **Generation Buffer**

Contents: One bottle containing buffer and a non-toxic antibiotic.
Format: Ready to Use
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.

8. **Phenylmethylsulfonyl fluoride**

Contents: Two tubes containing phenylmethylsulfonyl fluoride (PMSF).
Format: Concentrated; Requires Preparation
Volume: 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: 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.

9. **TMB Substrate**

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

10. **Stopping Solution**

Contents: One bottle containing 1M sulfuric acid.
Format: Ready to Use
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.

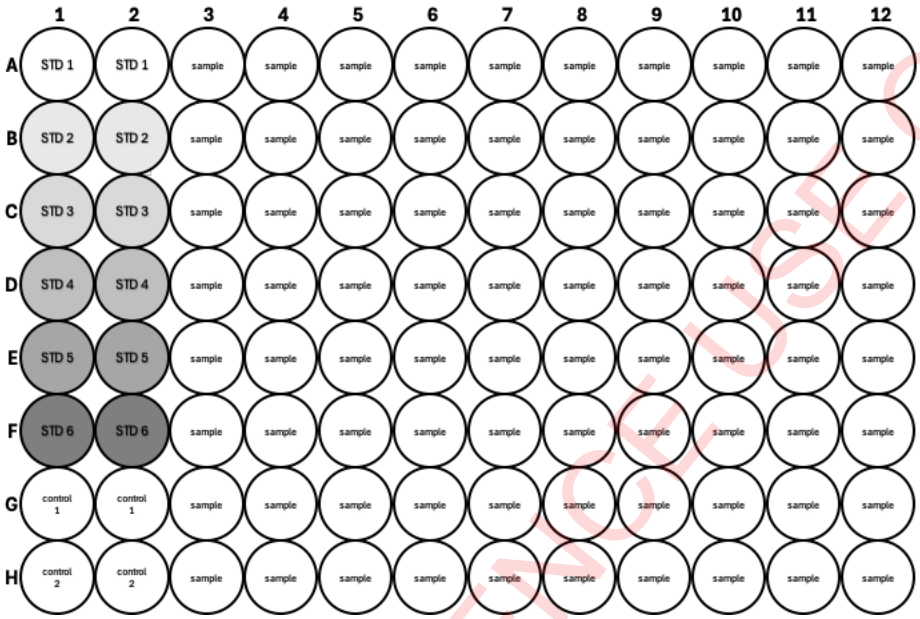
11. **Wash Buffer Concentrate (10x)**

Contents: Two bottles containing buffer with a non-ionic detergent and a non-mercury preservative
Format: Concentrated; Requires Preparation
Volume: 50 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 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: **Dilute 1:10** in distilled or deionized water before use. If the whole microplate is to be used dilute 50 mL of the wash buffer concentrate in 450 mL of distilled or deionized water.

RECOMMENDED ASSAY LAYOUT*



***** *Layout subject to change based on standard and control quantities*

ASSAY PROCEDURE

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.
3. **Prepare** all specimen samples that will be tested. Refer to section *Specimen Pre-Treatment & Storage.* For each plasma sample, both 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 *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.
    a **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.

b **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 HRP Conjugate 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 the addition of 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.

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 mean optical density values and the calibrator 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 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.

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 calibrator/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.

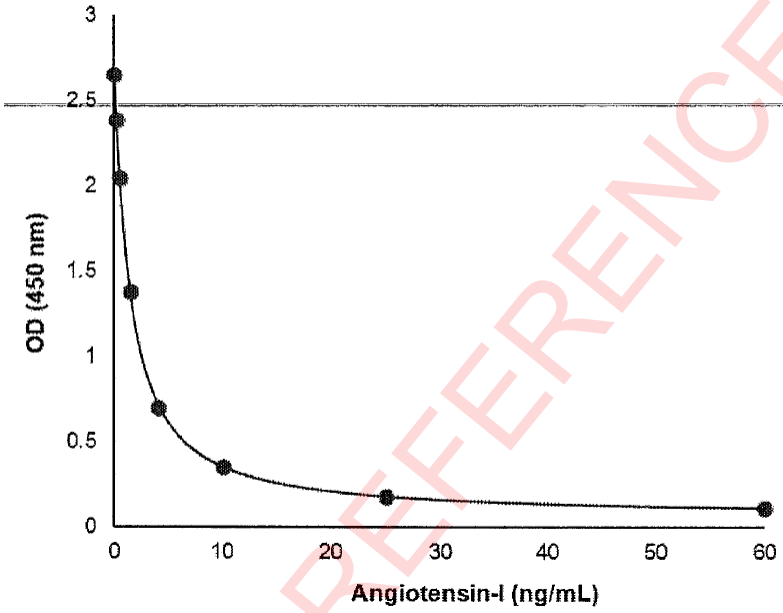
TYPICAL DATA

Sample data only. Do not use to calculate results.

Standard	Mean OD (450 nm)	% Binding	Value (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

TYPICAL STANDARD CURVE

Sample curve only. Do not use to calculate results.



WARRANTY INFORMATION

Eagle Biosciences, Inc. warrants its Product(s) to operate or perform substantially in conformance with its specifications, as set forth in the accompanying package insert. This warranty is expressly limited to the refund of the price of any defective Product or the replacement of any defective Product with new Product. This warranty applies only when the Buyer gives written notice to the Eagle Biosciences within the expiration period of the Product(s) by the Buyer. In addition, Eagle Biosciences has no obligation to replace Product(s) as result of a) Buyer negligence, fault, or misuse, b) improper use, c) improper storage and handling, d) intentional damage, or e) event of force majeure, acts of God, or accident.

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For further information about this kit, its application or the procedures in this kit, please contact the Technical Service Team at Eagle Biosciences, Inc. at info@eaglebio.com or at 866-411-8023.