INTRODUCTION

Prorenin (Pro)renin receptor (P)RR is a common receptor protein between renin and prorenin. When prorenin binds to (P)RR, it achieves a binding ability to angiotensinogens and gains a catalytic activity for conversion from angiotensinogens to angiotensin I in comparable level with renin. Additionally, when the (P)RR is stimulated by binding to prorenin, its intracellular signaling progresses. Through this, (P)RR research is believed to have an important implication in building of new strategy for suppressing of overactivated tissue RAA (Renin-Angiotensin-Aldosterone) system. (P)RR is a 39 kDa, single transmembrane receptor protein and it is reported that 29 kDa soluble form is generated by cleavage of (P)RR with furin. Thus the quantitative assay of soluble (P)RR in blood or urine samples is expected to provide new knowledges to clarification of disease mechanisms and development of new tools for diseases.

This ELISA kit can measure concentration of soluble (pro)renin receptor in blood or urine samples.

5) Dilution of test sample

This test samples should be diluted with "4, EIA buffer" suitably. The pre-assay with several different dilutions will be recommended to determine the proper dilution of samples. *Urine samples have to be diluted by 10-fold with "4, EIA buffer" soon after collection.

3. Measurement procedure

All reagents shall be brought to room temperature approximately 30 minutes before use. Then mix it gently and completely before use. Make sure of no change in quality of reagents. The curve shall be prepared simultaneously with the measurement of test samples.

1) Determine wells for reagent blank. Put 100 μL each of "4, EIA buffer" into the wells.

2) Determine wells for test sample blank, test sample and diluted standard. Then, put 100 μL each of test sample blank (tube-8), test sample and dilutions of standard (tube-1-7) into the appropriate wells.

3) Incubate the preincubated plate overnight at 4°C after covering it with plate lid.

4) Wash the plate with the prepared wash buffer and remove all liquid.

5) Incubate the preincubated plate for 4 minutes at 4°C after covering it with plate lid.

6) Wash the plate with the prepared wash buffer and remove all liquid.

7) Take the required quantity of "6, Chromogen" into a disposable test tube. Then, pipette 100 μL from the test tube into every well. Please do not return "6, Chromogen" back to the test tube. The solution of Chromogen will turn blue.

8) Incubate the precoated plate for 30 minutes at room temperature (shielded). The solution of Chromogen will turn blue.

9) Add 100 μL of "7, Stop solution" to all wells. Mix the solution by tapping the side of precoated plate. The solution will turn yellow by addition of "7, Stop solution".

11) Remove any dirt or drop of water on the bottom of the precoated plate and confirm there is no bubble on the surface of the solution. Then, run the plate reader and conduct measurement at 450 nm against a reagent blank. The measurement shall be done within 30 minutes after addition of "7, Stop solution".

SPECIAL ATTENTION

1) Test samples should be measured soon after collection. For the storage of test samples, store them frozen and do not repeat freeze/thaw cycles. Thaw the test samples at a low temperature and mix them completely before measurement.

2) Test samples should be diluted with "4, EIA buffer" soon after collection and store them in buffer solution.

3) Duplicative measurement of test samples and standard is recommended. Use test samples in neutral pH range. The contamination of organic solvent may affect the measurement.

4) Use only wash buffer contained in this kit for washing the precoated plate.

5) Insufficient washing may lead to the failure in measurement.

6) Avoid contact of Chromogen with metals.

7) Measurement should be done within 30 minutes after addition of "7, Stop solution".

CALCULATION OF TEST RESULT

See following picture.

For research use only, not for use in diagnostic procedures.
1. Dilution linearity

- Curve through these points to construct the standard curve. Read the concentration for unknown samples from the standard curve.

   - The typical standard curve is shown above. This curve can not be used to derive test results. Please run a standard curve for each assay.

2. Added Recovery Assay

   - Specimen | Additive Amount (pg/mL) | Theoretical Value (pg/mL) | Measured Value (pg/mL) | %
   - 10%FBS added Medium (x4) | 1,500 | 2,489 | 2,496 | 100.3
   - Human Serum (x50) | 750 | 1,739 | 1,667 | 95.9
   - Human Plasma (EDTA) (x50) | 4,000 | 4,495 | 4,143 | 92.2
   - Human Urine (x10) | 2,000 | 2,485 | 2,342 | 93.9
   - Mouse Serum (BALB/c) (x50) | 1,000 | 1,495 | 1,364 | 91.2
   - Mouse Plasma (BALB/c) (EDTA) (x50) | 4,000 | 4,462 | 3,957 | 88.7
   - 4. Inter - Assay

<table>
<thead>
<tr>
<th>Specimen</th>
<th>Mean Value (pg/mL)</th>
<th>SD (pg/mL)</th>
<th>CV (%)</th>
<th>n</th>
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</table>
   | Rat Serum (x50) | 750 | 924 | 780 | 84.4
   | 188 | 362 | 304 | 84.0 |
   | 23 | 196 | 169 | 85.4 |
   | Rat Plasma (EDTA) (x50) | 750 | 924 | 780 | 84.4
   | 188 | 362 | 304 | 84.0 |
   | 23 | 196 | 169 | 85.4 |

6. Sensitivity

   - The sensitivity for this kit was determined using the guidelines under the National Committee for Clinical Laboratory Standards (NCCLS) Evaluation Protocols. (National Committee for Clinical Laboratory Standards Evaluation Protocols, SC1, (1989) Villanova, PA: NCCLS.)

PRECAUTION FOR INTENDED USE AND/OR HANDLING

1. All reagents should be stored at 2 - 8°C. All reagents shall be brought to room temperature approximately 30 minutes before use.
2. "3, Standard" is lyophilized products. Be careful to open this vial.
3. "7, Stop solution" is a strong acid substance. Therefore, be careful not to have your skin and clothes contact it.
4. Dispose used materials after rinsing them with large quantity of water.
5. Precipitation may occur in "2, Labeled antibody Conc.", "4, EIA buffer" or "8, Wash buffer Conc.", however, there is no problem in the performance.
6. Wash hands after handling reagents.
7. Do not mix the reagents with the reagents from a different lot or kit.
8. Do not use expired reagents.
9. This kit is for research purpose only. Do not use for clinical diagnosis.

STORAGE AND THE TERM OF VALIDITY

Storage Condition: 2 - 8°C

The expiry date is specified on outer box.

REFERENCE