Enzyme Immunoassay for
Human Prorenin

For Research Use Only

INTRODUCTION

The precursor to renin, prorenin is a glycosylated aspartic protease. Renin cleaves angiotensinogen to form angiotensin I which is then converted to angiotensin II by angiotensin-converting enzyme (ACE).

PRINCIPLES OF PROCEDURE

This assay consists of a microplate coated with anti-human prorenin antibody. Human prorenin in the sample will bind to the antibody on the plate. A primary antibody to human prorenin is then added followed by a HRP conjugated secondary antibody. TMB is added as a detection reagent and the plate is read at 450nm. The OD is directly proportional to prorenin concentration.

MATERIALS PROVIDED

Component	Contents	Quantity	Storage	Cat. No.
Coated Plate	Anti-human prorenin coated 96-well plate	1 plate	4°C	RN35a
Zero Standard	Lyophilized plasma, prorenin and renin free	2 vials	4°C	RN35b
20 ng Standard	Lyophilized plasma containing 20 ng prorenin	1 vial	4°C	RN35c
Wash Buffer	10x solution for washing plate	50 mL	4°C	RN35d
Substrate	TMB Substrate	10 mL	4°C	RN35e
Primary Antibody	Anti-human prorenin antibody	1 vial	4°C	RN35f
Secondary Antibody	HRP conjugated antibody	1 vial	4°C	RN35g

MATERIALS NEEDED BUT NOT PROVIDED

1. Adjustable pipettes (10-1,000 µL) and disposable tips
2. Microplate reader with 450 nm filter
3. Deionized Water
4. 1 M Sulfuric Acid
5. TBS Buffer
6. Blocking Buffer

STORAGE CONDITIONS

1. Store this kit and its components at 4°C until use.
2. Once reconstituted, standards and antibodies can be stored at -70°C for future use. Avoid repeated freeze/thaw cycles.

WARNINGS AND PRECAUTIONS

1. Use aseptic technique when opening and dispensing reagents.
2. This kit is designed to work properly as provided and instructed. Additions, deletions, or substitutions to the procedure or reagents are not recommended, as they may be detrimental to the assay.
3. Exercise universal precautions during the performance or handling of this kit or any component contained therein.

PROCEDURAL NOTES

1. Human plasma prorenin concentrations have been reported previously. Averages range from 109 pg/mL[1] to 173 pg/mL[2]. Increased prorenin levels are seen in pregnancy and diabetes.

SAMPLE COLLECTION AND PREPARATION

Samples of human plasma and serum may be applied directly to the plate. Other sample types such as tissue extract, cell culture media, or urine should be tested on our RN36 assay.

REAGENT PREPARATION

1. **10x Wash Buffer:** Dilute the 50 mL of concentrate to 1x with 450 mL of DI water prior to use.
2. **TBS Buffer:** 0.1 M Tris, 0.15 M NaCl, pH 7.4.
3. **3% BSA Blocking Buffer:** 3% BSA in TBS Buffer.
4. **Standards (0 and 20 ng):** Reconstitute each standard as directed on the vials. Prepare immediately before use.
5. **Primary Antibody:** Reconstitute with 3% BSA Blocking Buffer as directed on the vial and vortex gently to mix. Prepare immediately prior to use.
6. **Secondary Antibody:** Dilute with 3% BSA Blocking Buffer as directed on the vial and vortex gently to mix. Prepare immediately prior to use.

STANDARD PREPARATION

Reconstitute the Standard as directed on the vial to give a 100 ng/mL Standard Stock Solution. **Do not prepare standards until you are ready to apply them to the plate.** Prepare the standard dilutions according to Table 1 below.

Table 1: Preparation of Standard Curve

Standard	Prorenin Concentration (ng/mL)	Zero Standard (μL)	Transfer Volume (μL)	Transfer Source	Final Volume (μL)
S_{10}	10	250	250	20 ng/mL Stock	250
S_9	5	250	250	S_{10}	300
S_8	2	300	200	S_9	250
S_7	1	250	250	S_8	250
S_6	0.5	250	250	S_7	300
S_5	0.2	300	200	S_6	250
S_4	0.1	250	250	S_5	250
S_3	0.05	250	250	S_4	300
S_2	0.02	300	200	S_3	250
S_1	0.01	250	250	S_2	500
S_0	0	500	---	---	500

ASSAY PROCEDURE

1. Add 100 μL of Standards or Unknowns to each well in duplicate. See Scheme I for a sample plate layout. Shake plate at 300 RPM for 30 minutes at room temperature (RT).
2. Wash wells according to the following wash procedure:
   a. Remove the contents of each well by inversion of the plate.
   b. Tap out the remaining contents of the plate onto a lint free paper towel.
   c. Add 300 μL of 1x Wash Buffer.
   d. Let stand for 2-3 minutes.
   e. Repeat procedure two more times, then proceed to step "f".
   f. Remove the contents of each well by inversion of plate into an appropriate disposal device.
   g. Tap out the remaining contents of the plate onto a lint free paper towel, then proceed to step 4.
3. Add 100 μl of the Primary Antibody to each well. Shake the plate at 300 rpm for 30 minutes at RT.
4. Wash the plate three times as in step 2.
5. Add 100 μl of the Secondary Antibody to each well. Shake the plate at 300 rpm for 30 minutes at RT.
6. Wash the plate three times as in step 2.
7. Add 100 μl of TMB Substrate to each well. Shake the plate at 300 rpm for 10-20 minutes at RT.
8. Stop the reaction by adding 50 μl of 1 N H_2SO_4 to each well and read the plate at 450 nm.

Scheme I:

	1	2	3	4	5	6	7	8	9	10	11	12
A	S_{10}	S_9	S_8	S_7	S_6	S_5	S_4	S_3	S_2	S_1	S_0	U_1
B	S_{10}	S_9	S_8	S_7	S_6	S_5	S_4	S_3	S_2	S_1	S_0	U_1
C	U_2	U_3	U_4	U_5	U_6	U_7	U_8	U_9	U_{10}	U_{11}	U_{12}	U_{13}
D	U_2	U_3	U_4	U_5	U_6	U_7	U_8	U_9	U_{10}	U_{11}	U_{12}	U_{13}
E	U_{14}	U_{15}	U_{16}	U_{17}	U_{18}	U_{19}	U_{20}	U_{21}	U_{22}	U_{23}	U_{24}	U_{25}
F	U_{14}	U_{15}	U_{16}	U_{17}	U_{18}	U_{19}	U_{20}	U_{21}	U_{22}	U_{23}	U_{24}	U_{25}
G	U_{26}	U_{27}	U_{28}	U_{29}	U_{30}	U_{31}	U_{32}	U_{33}	U_{34}	U_{35}	U_{36}	U_{37}
H	U_{26}	U_{27}	U_{28}	U_{29}	U_{30}	U_{31}	U_{32}	U_{33}	U_{34}	U_{35}	U_{36}	U_{37}

CALCULATIONS

1. Average the Zero Standard (S_0) absorbance values and subtract this average from the value obtained for all other wells. Most modern microplate readers are capable of doing this automatically.
2. Average the corrected absorbance for each pair of standards and unknowns.
3. Plot the average corrected absorbance for each standard against its concentration.
4. Using linear regression, plot a straight line and determine the equation for the standard curve.
5. Use the resulting equation to determine the concentration of the unknown samples. If the samples were diluted, remember to multiply by the dilution factor.

REFERENCES

1. Yokota, H *et al.*; (2005) Br. J. Ophthalmol.: **89**:871-873
2. Toffelmire, EB *et al.*; (1989) J Clin Invest.: **83**:679-687

DISCLAIMER

TECHNICAL SUPPORT

If you need technical information or assistance with assay procedures, please call our Technical Support Department at 1-800-692-4633 or 1-248-852-8815. Our staff will answer your questions about this or any other product in the Oxford Biomedical line.

GUARANTEE AND LIMITATION OF REMEDY

Oxford Biomedical Research, Inc. makes no guarantee of any kind, expressed or implied, which extends beyond the description of the material in this ELISA kit, except that these materials and this kit will meet our specifications at the time of delivery. Buyer's remedy and Oxford Biomedical Research, Inc.'s sole liability hereunder is limited to, at Oxford Biomedical Research, Inc.'s option, refund of the purchase price of, or the replacement of, material that does not meet our specification. By acceptance of our products, Buyer indemnifies and holds Oxford Biomedical Research, Inc. harmless against, assumes all liability for the consequence of its use or misuse by the Buyer, its employees, or others. Said refund or replacement is conditioned of Buyer notifying Oxford Biomedical Research, Inc. within (30) days of the receipt of product. Failure of Buyer to give said notice within said thirty (30) days shall constitute a waiver by the Buyer of all claims hereunder with respect to said material(s).

Oxford Biomedical Research, Inc.
P.O. Box 522
Oxford, MI 48371 U.S.A.

Orders: 800-692-4633
Technical Service: 248-852-8815
Fax: 248-852-4466
E-mail: info@oxfordbiomed.com

Made in the U.S.A.