ab157525 – Prorenin Human ELISA Kit

Instructions for Use

For the quantitative determination of Prorenin in Human plasma.

This product is for research use only and is not intended for diagnostic use.

Version 1 Last Updated 15 April 2013

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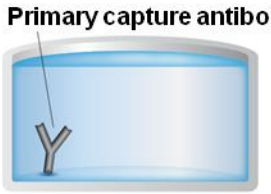
1. BACKGROUND

Abcam's Prorenin ELISA (Enzyme-Linked Immunosorbent Assay) kit is designed for is for the quantitative determination of prorenin in Human plasma. Active renin will not be detected by this assay. Prorenin is measured directly by ELISA without pre-treatment of samples or conversion to renin.

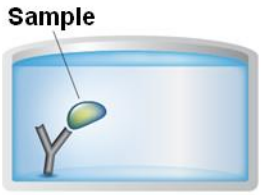
Prorenin is a glycosylated aspartic protease that consists of 2 homologous lobes and is the precursor of renin. Renin activates the renin-angiotensin system by cleaving angiotensinogen, produced by the liver, to yield angiotensin I, which is further converted into angiotensin II by ACE, the angiotensin-converting enzyme primarily within the capillaries of the lungs. It has been reported that the levels of circulating prorenin (but not renin) are increased in diabetic subjects.

Human prorenin will bind to the capture antibody coated on the microtiter plate. After appropriate washing steps, anti-Human prorenin primary antibody binds to the captured protein. Only prorenin and not active renin will be detected by the primary antibody. Excess antibody is washed away and bound primary antibody is then reacted with the secondary antibody conjugated to horseradish peroxidase. TMB substrate is used for color development at 450nm. A standard calibration curve is prepared along with the samples to be measured using dilutions of prorenin. The amount of color development is directly proportional to the concentration of prorenin in the sample.

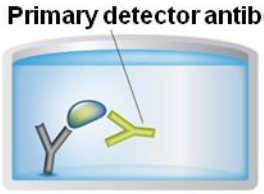
2. ASSAY SUMMARY



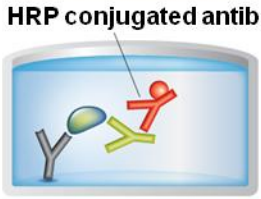
Remove appropriate number of antibody coated well strips. Equilibrate all reagents to room temperature. Prepare all the reagents, samples, and standards as instructed.



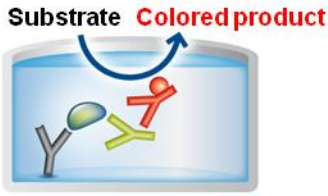
Add standard or sample to each well used. Incubate at room temperature.



Aspirate and wash each well. Add primary detector antibody. Incubate at room temperature.



Aspirate and wash each well. Add HRP conjugated antibody to each well. Incubate at room temperature.



Aspirate and wash each well. Add TMB Substrate to each well. Immediately begin recording the color development

3. PRECAUTIONS

Please read these instructions carefully prior to beginning the assay.

- Do not mix any reagents or components of this kit with any reagents or components of any other kit. This kit is designed to work properly as provided.
- Always pour peroxidase substrate out of the bottle into a clean test tube. Do not pipette out of the bottle as contamination could result.
- Keep plate covered except when adding reagents, washing, or reading.
- DO NOT pipette reagents by mouth and avoid contact of reagents and specimens with skin.
- DO NOT smoke, drink, or eat in areas where specimens or reagents are being handled.
- The Prorenin activity standards are of Human origin. Each donor unit has been tested and found negative for the presence of HBsAg, anti-HIV 1+2, anti-HBc, and anti-HCV. Since no tests are currently available to assure that no infectious agents are present, the plasma must be treated as potentially hazardous.

4. STORAGE AND STABILITY

Store kit at 2-8ºC immediately upon receipt.

Refer to list of materials supplied for storage conditions of individual components. Observe the storage conditions for individual prepared components in section 8. Reagent Preparation.

5. MATERIALS SUPPLIED

Item	Amount	Storage Condition (Before Preparation)
Prorenin Coated Microplate	96 Wells	2-8ºC
10X Wash Buffer	50 mL	2-8ºC
Human Prorenin Negative Control (0 ng)	2 Vials	2-8ºC
Human Prorenin Positive Control (20 ng)	1 Vial	2-8ºC
Anti-Human Prorenin Primary Antibody (lyophilized)	1 Vial	2-8ºC
Anti-mouse HRP Antibody	1 Vial	-80ºC
TMB Substrate Solution	10 mL	2-8ºC

6. MATERIALS REQUIRED, NOT SUPPLIED

These materials are not included in the kit, but will be required to successfully utilize this assay:

- Microtiter plate shaker capable of 300 rpm uniform horizontally circular movement.
- Manifold dispenser/aspirator or automated microplate washer.
- Microplate reader capable of measuring absorbance at 450 nm.
- Pipettes and Pipette tips.
- Deionized or distilled water.
- Polypropylene tubes for dilution of standard.
- Paper towels or laboratory wipes.
- 1N H_2SO_4 or 1N HCl.
- Bovine Serum Albumin Fraction V (BSA).
- Tris(hydroxymethyl)aminomethane (Tris).
- Sodium Chloride (NaCl).

7. TECHNICAL HINTS

- Samples generating values higher than the highest standard should be further diluted in the appropriate sample dilution buffers.
- Avoid foaming or bubbles when mixing or reconstituting components.
- Avoid cross contamination of samples or reagents by changing tips between sample, standard and reagent additions.
- Ensure plates are properly sealed or covered during incubation steps.
- Complete removal of all solutions and buffers during wash steps.
- **This kit is sold based on number of tests. A 'test' simply refers to a single assay well. The number of wells that contain sample, control or standard will vary by product. Review the protocol completely to confirm this kit meets your requirements. Please contact our Technical Support staff with any questions.**

8. REAGENT PREPARATION

Equilibrate all reagents and samples to room temperature (18-25°C) prior to use.

8.1 **1X Tris-Buffered Saline (TBS)**

0.1M Tris, 0.15M NaCl, pH 7.4.

8.2 **1X Blocking Buffer**

3% BSA (w/v) in 1X TBS.

8.3 **1X Wash Buffer**

Dilute 50 mL of 10X Wash Buffer concentrate with 450 mL of deionized water. Mix gently and thoroughly.

8.4 **1X Prorenin Primary Antibody**

Reconstitute Anti-Human Prorenin Primary Antibody to prepare a 1X Prorenin Primary Antibody by adding 10 mL of 1X Blocking Buffer directly to the vial and agitate gently to completely dissolve contents.

8.5 **1X HRP Antibody**

Prepare 1X HRP Antibody by diluting 3 µL of Anti-mouse HRP Antibody in 10 mL of 1X Blocking Buffer.

- Reconstituted primary antibody may be stored at -80°C for later use. Do not freeze-thaw the primary antibody more than once.

9. STANDARD PREPARATION

Prepare serially diluted standards immediately prior to use. Always prepare a fresh set of standards for every use.

9.1 Reconstitute Human Prorenin Positive Control (20 ng) by adding 1 mL of Deionized water to each vial and agitate gently to completely dissolve contents. Final concentration is 20 ng/mL.

9.2 Reconstitute Human Prorenin Negative Control (0 ng) by adding 1.5 mL of deionized water to each vial and agitate gently to completely dissolve contents**.**

9.3 Label ten tubes # 1-10.

9.4 Prepare **Standard #1,** in tube #1 by adding 250 µL Human Prorenin Positive Control (20 ng/mL) to 250 µL Human Prorenin Negative Control (0 ng/mL) and mix gently and thoroughly.

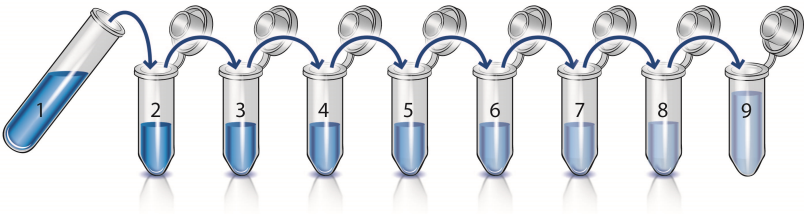
9.5 Prepare **Standard #2,** in tube #2 by adding 250 µL **Standard #1** to 250 µL Human Prorenin Negative Control (0 ng/mL) and mix gently and thoroughly.

9.6 Prepare **Standard #3,** in tube #3 by adding 200 µL **Standard #2** to 300 µL Human Prorenin Negative Control (0 ng/mL) and mix gently and thoroughly.

9.7 Using the table below as a guide, prepare further serial dilutions.

9.8 Human Prorenin Negative Control (0 ng/mL) serves as the zero standard, 0 ng/mL (Tube#10).

Standard #	Sample to dilute	Volume to dilute (µL)	Human Prorenin Negative Control (0 ng/mL) (µL)	Total Volume (µL)	Starting Conc. (ng/mL)	Final Conc. (ng/mL)
1	Human Prorenin Positive Control (20 ng/mL)	250	250	500	20	10
2	Standard #1	250	250	500	10	5
3	Standard #2	200	300	500	5	2
4	Standard #3	250	250	500	2	1
5	Standard #4	250	250	500	1	0.5
6	Standard #5	200	300	500	0.5	0.2
7	Standard #6	250	250	500	0.2	0.1
8	Standard #7	250	250	500	0.1	0.05
9	Standard #8	200	300	500	0.05	0.02
10	-	-	500	500	0	0



- Reconstituted standards may be stored at -80°C for later use. Do not freeze-thaw the standard more than once.

10. SAMPLE COLLECTION AND STORAGE

- **Plasma** – Collect plasma using EDTA or citrate as an anticoagulant. Centrifuge for 15 minutes at 1000 x g within 30 minutes of collection. Assay immediately or aliquot and store at ≤ -20°C. Avoid repeated freeze-thaw cycles.

11. PLATE PREPARATION

- The 96 well plate strips included with this kit are supplied ready to use. It is not necessary to rinse the plate prior to adding reagents.
- Unused well strips should be returned to the plate packet and stored at 4°C.
- For each assay performed, a minimum of two wells must be used as blanks, omitting primary antibody from well additions.
- For statistical reasons, we recommend each sample should be assayed with a minimum of two replicates (duplicates).
- Well effects have not been observed with this assay. Contents of each well can be recorded on the template sheet included in the Resources section.

12. ASSAY PROCEDURE

- **Equilibrate all materials and prepared reagents to room temperature prior to use.**
- **It is recommended to assay all standards, controls and samples in duplicate.**

12.1. Add 100 µL prepared standards (in duplicate) and samples to wells.

12.2. Shake plate at 300 rpm for 30 minutes.

12.3. Wash wells three times with 300 µL 1X Wash Buffer. Remove excess wash by gently tapping plate on paper towel.

12.4. Add 100 µL of 1X Prorenin Primary Antibody primary antibody to all wells.

12.5. Shake plate at 300 rpm for 30 minutes.

12.6. Wash wells three times with 300 µL 1X Wash Buffer. Remove excess wash by gently tapping plate on paper towel.

12.7. Add 100 µL of 1X HRP Antibody to all wells.

12.8. Shake plate at 300 rpm for 30 minutes.

12.9. Wash wells three times with 300 µL 1X Wash Buffer. Remove excess wash by gently tapping plate on paper towel.

12.10. Add 100 µL TMB Substrate Solution to all wells and shake plate for 3-8 minutes. Substrate will change from colorless to different strengths of blue.

12.11. Quench reaction by adding 50 µL of 1N H_2SO_4 or HCl stop solution to all wells when samples are visually in the same range as the standards. Add stop solution to wells in the same order as substrate upon which color will change from blue to yellow. Mix thoroughly by gently shaking the plate.

12.12. Set the absorbance at 450 nm in a microtiter plate spectrophotometer. Measure the absorbance in all wells at

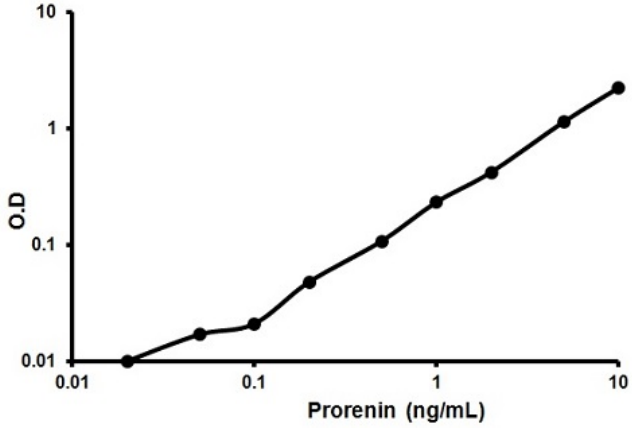
450 nm. Subtract zero point from all standards and unknowns to determine corrected absorbance (A450).

13. CALCULATIONS

Plot A_{450} against the amount of Prorenin in the standards. Fit a straight line through the linear points of the standard curve using a linear fit procedure if unknowns appear on the linear portion of the standard curve. Alternatively, create a standard curve by analyzing the data using a software program capable of generating a four parameter logistic (4PL) curve fit. The amount of Prorenin in the unknowns can be determined from this curve. If samples have been diluted, the calculated concentration must be multiplied by the dilution factor.

14. TYPICAL DATA

TYPICAL STANDARD CURVE – Data provided for **demonstration purposes only**. A new standard curve must be generated for each assay performed.



Standard Curve Measurements	
Conc. (ng/mL)	**O.D.**
0	0
0.02	0.010
0.05	0.017
0.1	0.021
0.2	0.048
0.5	0.108
1	0.234
2	0.424
5	1.147
10	2.253

15. TYPICAL SAMPLE VALUES

SENSITIVITY -

The minimum detectable dose (MDD) was determined by adding two standard deviations to the mean optical density value of twenty zero standard replicates (range OD_{450}: 0.066 - 0.074) and calculating the corresponding concentration. The MDD was 0.013 ng/mL.

RECOVERY –

(Sample spiking at a range of concentrations in representative sample matrices)

Sample Type	Average % Recovery	Range
1	102	86 -122%
2	93	90 - 97%
3	101	95 - 106%
4	103	99 - 108%

LINEARITY OF DILUTION -

Plasma Dilution	% Expected Value
1:2	96
1:4	102
1:8	107
1:16	119

PRECISION –

	Intra-Assay	Inter-Assay
n =	20	10
Mean Sample Conc. (ng/mL)	0.345	0.342
SD	0.020	0.045
%CV	5.74	13.2

16. ASSAY SPECIFICITY

This assay recognizes recombinant and natural Human prorenin. The factors listed below were prepared at 10 ng/mL in depleted plasma and assayed for cross-reactivity. No significant cross-reactivity was observed with:

Recombinant Human renin

Recombinant Mouse Prorenin

Recombinant Rat Prorenin.

17. TROUBLESHOOTING

Problem	Cause	Solution
Poor standard curve	Inaccurate pipetting	Check pipettes
	Improper standards dilution	Prior to opening, briefly spin the stock standard tube and dissolve the powder thoroughly by gentle mixing
Low Signal	Incubation times too brief	Ensure sufficient incubation times; change to overnight standard/sample incubation
	Inadequate reagent volumes or improper dilution	Check pipettes and ensure correct preparation
Large CV	Plate is insufficiently washed	Review manual for proper wash technique. If using a plate washer, check all ports for obstructions
	Contaminated wash buffer	Prepare fresh wash buffer
Low sensitivity	Improper storage of the ELISA kit	Store the reconstituted protein at -80°C, all other assay components 4°C. Keep substrate solution protected from light.

18. NOTES

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