Total Antigen ELISA for
Rat Prorenin/Renin

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INTRODUCTION

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 (1).

PRINCIPLES OF PROCEDURE

This ELISA uses a capture antibody coated to the 96-well plate to bind Rat Prorenin and Renin. A detection antibody conjugated to biotin is then applied. After washing, avidin conjugated to horseradish peroxidase (HRP) is applied. TMB substrate is added for color development which is proportional to the total concentration of prorenin and rennin in the sample. Sample concentrations can be determined by comparing OD values to the standard curve.

MATERIALS PROVIDED

Component	Contents	Quantity	Storage	Cat. No.
Coated Plate	Anti-Rat Prorenin/Renin	1 vial	4°C	RN46a
Standard	Rat Prorenin (lyophilized)	1 vial	4°C	RN46b
Wash Buffer	10x solution for washing plate	50 mL	4°C	RN46c
Primary Antibody	Anti Rat Prorenin/Renin Antibody	1 vial	4°C	RN46d
Avidin HRP	HRP Labeled Avidin	1 vial	4°C	RN46e
Substrate	TMB Substrate	10 mL	4°C	RN46f

MATERIALS NEEDED BUT NOT PROVIDED

1. Pipettes covering 0-10 μl and 200-1000 μl and tips
2. 12-channel pipette covering 30-300μl
3. 1N Sulfuric Acid (H_2SO_4)
4. Deionized Water (DI water)
5. Microtiter plate spectrophotometer with a 450 nm filter
6. Microtiter plate shaker with uniform horizontally circular movement up to 300 rpm

STORAGE CONDITIONS

1. Store this kit and its components at 4°C until use.
2. Reconstituted standards and primary may be stored at -70°C for later use. **DO NOT** freeze/thaw the Standards and Primary Antibody more than once.

PROCEDURAL NOTES

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.

SAMPLE COLLECTION AND PREPARATION

Typical rat prorenin levels range from 0 to 400 ng/mL. The assay range for this ELISA is 0.1 to 100 ng/mL. Samples with prorenin levels above 100 ng/mL should be diluted in Blocking Buffer and retested.

REAGENT PREPARATION

The following solutions should be prepared fresh before starting the assay.

1. **TBS Buffer:** 0.1 M TRIS, 0.15 M NaCl, pH 7.4
2. **3% BSA Blocking Buffer:** 3% BSA in TBS Buffer.
3. **10x Wash Buffer:** Dilute the 50 mL of concentrate to 1x with 450 mL of DI water prior to use.
4. **Standard:** Reconstitute with 1.0 mL of 3% BSA Blocking Buffer and vortex gently to mix. Prepare according to included Standard Dilution Table immediately prior to use.
5. **Primary Antibody:** Reconstitute with 10 mL of 3% BSA Blocking Buffer and vortex gently to mix. Prepare immediately prior to use.
6. **Avidin HRP:** Dilute with 10 mL of 3% BSA Blocking Buffer and vortex gently to mix. Prepare immediately prior to use.

STANDARD PREPARATION

Reconstitute the Standard as directed on the vial to give a 1000 ng/mL Standard Stock Solution (see Reagent Preparation). **Do not prepare the standards until you are ready to apply them to the plate.**

Table 1: Preparation of Standard Curve

Standard	Prorenin Concentration (ng/mL)	Blocking Buffer (μL)	Transfer Volume (μL)	Transfer Source	Final Volume (μL)
S_{10}	100	900	100	Stock	750
S_9	50	250	250	S_{10}	300
S_8	20	300	200	S_9	250
S_7	10	250	250	S_8	250
S_6	5	250	250	S_7	300
S_5	2	300	200	S_6	250
S_4	1	250	250	S_5	250
S_3	0.5	250	250	S_4	300
S_2	0.2	300	200	S_3	250
S_1	0.1	250	250	S_2	500
S_0	0	500	---	---	500

ASSAY PROCEDURE

1. Add 100 μl of the Standards and unknowns to the wells in duplicate. Shake the plate at 300 rpm for 30 minutes at room temperature (RT). For a suggested plate layout, see **Scheme I** below.
2. Wash the plate 3 times 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 3.
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 300rpm 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 2-10 minutes at RT.
8. Stop the reaction by adding 50 μl of 1N H_2SO_4 to each well and read the plate at 450 nm.

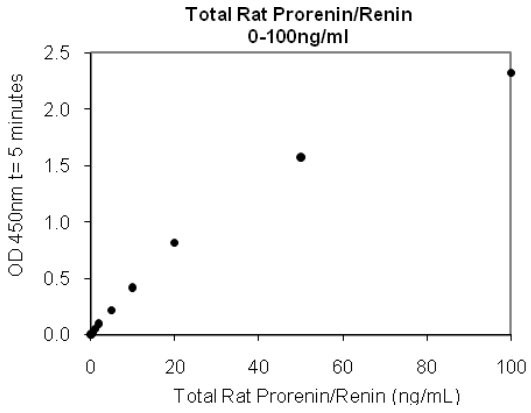
Scheme I: Sample Plate Layout

	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. Plot the A_{450} against the concentration of uPA in the standards.
2. Fit a straight line through the points using a linear fit procedure.
3. Calculate the uPA concentrations in the unknowns using the equation generated by the standard curve.

Figure 1: Typical Standard Curve



EXPECTED VALUES

Rat prorenin levels range from 0-400 ng/ml depending on assay methodology[2]. Human plasma levels of prorenin are greater in males than females and correlate positively with age and negatively with blood pressure[3]. Plasma and serum concentrations increase in several conditions such as pregnancy, progressive diabetes mellitus, diabetes mellitus with microvascular disease, and diabetic retinopathy[4,5].

REFERENCES

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