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Total Antigen ELISA for Rat Prorenin/Renin

Catalog Number: PRO29-K01

For Research Use Only. Not for use in diagnostic procedures.

v. 2 (26 SEP 24)

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INTENDED USE

The Rat Prorenin & Renin ELISA Assay kit is for the quantitative determination of Rat Prorenin and Renin level in biological fluids by an ELISA (Enzyme-Linked Immunosorbent Assay). The Rat Prorenin & Renin ELISA Assay is for research use only and not intended for use 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.

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.

PRINCIPLE OF THE ASSAY

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.

Component	Contents	Quantity	Storage
Coated Plate	Anti-Rat Prorenin/Renin	1 Vial	4°C
Standard	Rat Prorenin (lyophilized)	1 Vial	4°C
Wash Buffer	10x Solution for washing plate	50mL	4°C
Primary Antibody	Anti Rat Prorenin/ Renin Antibody	1 Vial	4°C
Avidin HRP	HRP labeled Avidin	1 Vial	4°C
Substrate	TMB Substrate	10 mL	10°C

MATERIALS REQUIRED BUT NOT PROVIDED

1. Pipettes covering 0-10 µl and 200-1000 µl and tips
2. 12-channel pipette covering 30-300µl
3. 1 N Sulfuric Acid (H₂SO₄)
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 COLLECITON 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	S3	250
S ₁	0.1	250	250	S2	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 H₂SO₄ to each well and read the plate at 450 nm.

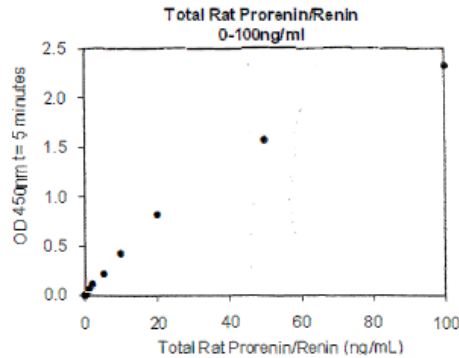
Scheme I: Sample Plate Layout

	1	2	3	4	5	6	7	8	9	10	11	12
A	S10	S9	S8	S7	S6	S5	S4	S3	S2	S1	S0	U1
B	S10	S9	S8	S7	S6	S5	S4	S3	S2	S1	S0	U1
C	U2	U3	U4	U5	U6	U7	U8	U9	U10	U11	U12	U13
D	U2	U3	U4	U5	U6	U7	U8	U9	U10	U11	U12	U13
E	U14	U15	U16	U17	U18	U19	U20	U21	U22	U23	U24	U25
F	U14	U15	U16	U17	U18	U19	U20	U21	U22	U23	U24	U25
G	U26	U27	U28	U29	U30	U31	U32	U33	U34	U35	U36	U37
H	U26	U27	U28	U29	U30	U31	U32	U33	U34	U35	U36	U37



CALCULATIONS

1. Plot the A450 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.



EXPECTED VALUES

Rat prorenin levels range from 0-400 ng/ml depending on assay methodology". Human plasma levels of prorenin are greater in males than females and correlate positively with age and negatively with blood pressure³ Plasma and serum concentrations increase in several conditions such as pregnancy, progressive diabetes mellitus, diabetes mellitus with microvascular disease, and diabetic retinopathy.

REFERENCES

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