

Rat Prorenin/Renin Total Antigen Assay

Strip well format. Reagents for up to 96 tests.

For Research Use Only.

INTENDED USE

This rat prorenin/renin total antigen assay is intended for the quantitative determination of total prorenin and renin antigen in rat plasma and serum.

BACKGROUND

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].

ASSAY PRINCIPLE

Rat prorenin and renin will bind to the affinity purified capture antibody coated on the microtiter plate. After appropriate washing steps, biotin labeled polyclonal anti-rat prorenin primary antibody binds to the captured protein. Excess antibody is washed away and bound polyclonal antibody is reacted with avidin 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 rat prorenin. Color development is proportional to the total concentration of prorenin and renin in the samples.

REAGENTS PROVIDED

◆ **Immunoassay plate:**

1-96 well immulon plate (8X12 removable wells) coated, blocked, and dried with prorenin capture antibody

◆ **10X Wash Buffer:**

1 bottle of 50ml; bring to 1X using DI water

◆ **Rat prorenin antigen standard:**

1 vial of lyophilized standard

◆ **Anti-rat prorenin primary antibody:**

1 vial of lyophilized biotin labeled polyclonal antibody

◆ **Avidin peroxidase secondary reagent:**

1 vial of concentrated HRP labeled avidin

◆ **TMB substrate solution:**

1 bottle of 10ml solution

STORAGE AND STABILITY

All kit components must be stored at 4°C. Store unopened plate and any unused microtiter strips in the pouch with desiccant. 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. All other unused kit components must be stored at 4°C. Kit should be used no later than the expiration date.

REAGENTS AND EQUIPMENT REQUIRED

- 1-channel pipettes covering 0-10µl and 200-1000µl
- 12-channel pipette covering 30-300µl
- Paper towels or kimwipes
- 50ml tubes, 1.5ml centrifuge tubes
- 1N H₂SO₄

- DI water
- Magnetic stirrer and stir-bars
- Plastic containers with lids
- Microtiter plate spectrophotometer operable at 450nm
- Microtiter plate shaker with uniform horizontally circular movement up to 300rpm.

WARNINGS

Warning – Avoid skin and eye contact when using TMB One substrate solution since it may be irritating to eyes, skin, and respiratory system. Wear safety goggles and gloves.

PRECAUTIONS

- 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.
- **DO NOT** pipette reagents by mouth.
- Always pour substrate out of the bottle into a clean test tube. **DO NOT** pipette out of the bottle as you could contaminate the substrate.
- Keep plate covered except when adding reagents, washing, or reading.
- **DO NOT** smoke, drink, or eat in areas where specimens or reagents are being handled.

PREPARATION OF REAGENTS

- TBS buffer:** 0.1M Tris 0.15M NaCl pH 7.4
- Blocking buffer:** 3% BSA in TBS buffer (BSA)

SPECIMEN COLLECTION

The assay measures total rat prorenin and renin in the 0.1-100 ng/ml range. Samples giving rat prorenin levels above 100ng/ml should be diluted in blocking buffer before use.

ASSAY PROCEDURE

Perform assay at room temperature. Vigorously shake plate (300rpm) at each step of the assay.

Preparation of Standard:

Reconstitute standard as directed on the vial to give a 1000ng/ml solution.

Dilution table for preparation of rat prorenin standards:

Prorenin concentration (ng/ml)	Dilutions
100	900µl (BSA) + 100µl (1000ng/ml)
50	250µl (BSA) + 250µl (100ng/ml)
20	300µl (BSA) + 200µl (50ng/ml)
10	250µl (BSA) + 250µl (20ng/ml)
5	250µl (BSA) + 250µl (10ng/ml)
2	300µl (BSA) + 200µl (5ng/ml)
1	250µl (BSA) + 250µl (2ng/ml)
0.5	250µl (BSA) + 250µl (1ng/ml)
0.2	300µl (BSA) + 200µl (0.5ng/ml)
0.1	250µl (BSA) + 250µl (0.2ng/ml)
0	500µl (BSA) Zero point to determine background

NOTE: DILUTIONS FOR THE STANDARD CURVE MUST BE MADE AND APPLIED TO THE PLATE IMMEDIATELY.

Standard and Unknown Addition:

Remove microtiter plate from bag. Add 100µl standards in duplicate and 100µl unknown samples to wells. Carefully record the position of standards and unknowns. Shake plate at 300rpm for 30 minutes. Wash wells three times with 300µl wash buffer. Remove excess wash by gently tapping plate on paper towel or kimwipe.

Primary Antibody Addition:

Add 10ml of 3% BSA blocking buffer directly to the primary antibody vial and agitate gently to completely dissolve contents. Add 100µl to all wells. Shake plate at 300rpm for 30 minutes. Wash wells three times with 300µl wash buffer. Remove excess wash by gently tapping plate on paper towel or kimwipe.

Secondary Antibody Addition:

Dilute 2µl into 10ml of 3% BSA blocking buffer and add 100µl to all wells. Shake plate at 300rpm for 30 minutes. Wash wells three times with 300µl wash buffer. Remove excess wash by gently tapping plate on paper towel or kimwipe.

Substrate Incubation:

Add 100µl of TMB substrate solution to all wells and shake plate at 300rpm for 5-15 minutes. Quench the reaction with the addition of 50µl of 1N H₂SO₄ and read final absorbance values at 450nm. NOTE: Time for substrate development is dependent on needs of researcher.

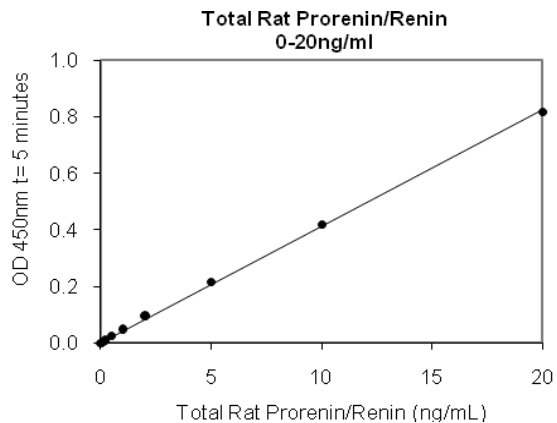
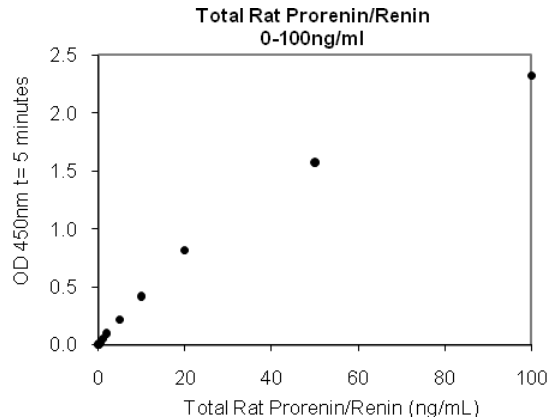
Measurement:

Set the absorbance at 450nm in a microtiter plate spectrophotometer. Measure the absorbance in all wells at 450nm. Subtract zero point from all standards and unknowns to determine corrected absorbance (A₄₅₀).

Assay Calibration:

Plot A₄₅₀ against the amount of rat prorenin in the standards. Fit a straight line through the points using a linear fit procedure. The amount of total rat prorenin and renin in the unknowns can be determined from this curve.

A typical standard curve.
(EXAMPLE ONLY, DO NOT USE)



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].

Plate Layout

	1	2	3	4	5	6	7	8	9	10	11	12
A	0	0.1ng/ml	0.2ng/ml	0.5ng/ml	1ng/ml	2.5ng/ml	5ng/ml	10ng/ml	20ng/ml	50ng/ml	100ng/ml	
B	0	0.1ng/ml	0.2ng/ml	0.5ng/ml	1ng/ml	2.5ng/ml	5ng/ml	10ng/ml	20ng/ml	50ng/ml	100ng/ml	
C												
D												
E												
F												
G												
H												

96 Well Plate

Standards: 22 wells

Samples: 74 wells

PERFORMANCE CHARACTERISTICS

The assay measures total rat prorenin and renin in the 0.1-100 ng/ml range.

DISCLAIMER

This information is believed to be correct but does not claim to be all-inclusive and shall be used only as a guide. The supplier of this kit shall not be held liable for any damage resulting from handling or from contact with the above product.

REFERENCES

1. Luetscher, JA *et al.*: Increased plasma inactive renin in diabetes mellitus. A marker of microvascular complications. *N. Engl J. Med.*, **312**:1412-1417, 1985.
2. Ioannou, P *et al.*: Activation and measurement of plasma prorenin in the rat. *Can. J. Physiol. Pharmacol.*, **69**(9): 1331-40, 1991.
3. Danser, AH *et al.*: Determinants of interindividual variation of renin and prorenin concentrations: evidence for a sexual dimorphism of (pro)renin levels in humans. *J. Hypertens.*, **16**:853-862, 1988.

4. Yokota, H *et al.*: Serum prorenin levels and diabetic retinopathy in type 2 diabetes: new method to measure serum level of prorenin using antibody activating direct kinetic assay. *Br. J. Ophthalmol.*, **89**:871-873, 2005.
5. Schmieder, RE: The potential role of prorenin in diabetic neuropathy. *J. Hypertens.*, **25**:1323-1326, 2007.