

Human Prorenin Direct Assay

Strip well format. Reagents for up to 96 tests.

For Research Use Only.

INTENDED USE

Human prorenin direct assay is intended for the quantitative determination of prorenin in biological fluids. Active renin will not be detected by this assay. Prorenin is measured directly by ELISA without pretreatment of samples or conversion to renin [1].

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 [2].

ASSAY PRINCIPLE

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.

REAGENTS PROVIDED

- ◆ **Coated plate:**
1-96 well immulon plate coated, blocked, and dried with capture antibody
- ◆ **10X Wash Buffer:**
1 bottle of 50ml wash buffer; bring to 1X using DI water
- ◆ **Human prorenin standard:**
1 vial of lyophilized standard
- ◆ **Anti-human prorenin primary antibody:**
1 vial of lyophilized antibody
- ◆ **Horseradish peroxidase conjugated secondary antibody:**
1 vial of concentrated labeled antibody
- ◆ **TMB substrate solution:**
1 bottle of 10 ml

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 20-200 µl, 500-5000 µl and 200-1000µl
- 12-channel pipette for 30-300µl
- Paper towels or kimwipes
- 1.5ml micro centrifuge tubes
- 1N H₂SO₄
- DI water
- Magnetic stirrer and stir-bars
- Plastic containers with lids
- TBS buffer

- Blocking buffer
- 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

SPECIMEN PREPARATION

Samples of human plasma, serum, cell culture media, or tissue extracts may be applied directly to the plate.

The assay measures human prorenin in the 0.005-1 ng/ml range.

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 vial to give a 20ng/ml standard solution.

Dilution table for preparation of human prorenin standards:

Prorenin concentration (ng/ml)	Dilutions
1	950µl (BSA) + 50µl (20ng/ml)
0.5	500µl (BSA) + 500µl (1ng/ml)
0.25	500µl (BSA) + 500µl (0.5ng/ml)
0.1	600µl (BSA) + 400µl (0.25ng/ml)
0.05	500µl (BSA) + 500µl (0.1ng/ml)
0.02	500µl (BSA) + 400µl (0.05ng/ml)
0.01	500µl (BSA) + 500µl (0.02ng/ml)
0.005	500µl (BSA) + 500µl (0.01ng/ml)

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 standard in duplicate and unknown to wells. Carefully record 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 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 TMB substrate to all wells and shake plate for 5-15 minutes. Quench the reaction by the addition of 50µl of 1M H₂SO₄ and read final absorbance values at 450nm.

NOTE: Time for substrate development is dependent on needs of researcher.

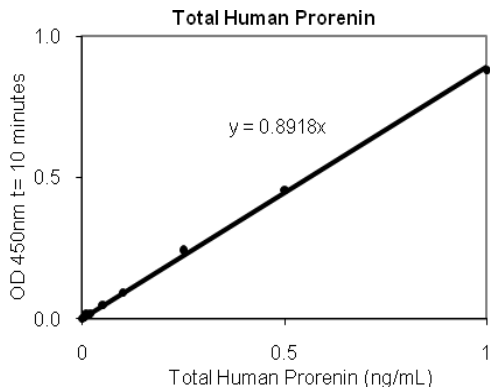
Measurement:

Set the absorbance at 450nm in a microtiter plate spectrophotometer. Measure the absorbance in all wells at 450nm, A₄₅₀.

Assay Calibration:

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

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



EXPECTED VALUES

Human plasma levels of prorenin are greater in males than females and correlate positively with age and negatively with blood pressure [3]. Serum concentrations increase with progressive diabetes mellitus and diabetic retinopathy [4]:

Disease State	Prorenin (ng/ml)	Standard Deviation
Control	0.1091	0.0661
No-DR	0.1946	0.1604
No-PDR	0.2715	0.2203
PDR	0.4284	0.3584

Control group: Normal, no diabetes.
No-DR group: Diabetes, no retinopathy.
No-PDR group: Non-proliferative retinopathy.
PDR group: Proliferative retinopathy.

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. Schalekamp, MADH *et al.*: Newly developed renin and prorenin assays and the clinical evaluation of renin inhibitors. *J. Hypertens.*, **26**:928-937, 2008.
2. Luetscher, JA *et al.*: Increased plasma inactive renin in diabetes mellitus. A marker of microvascular complications. *N. Engl J. Med.*, **312**:1412-1417, 1985.
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.

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