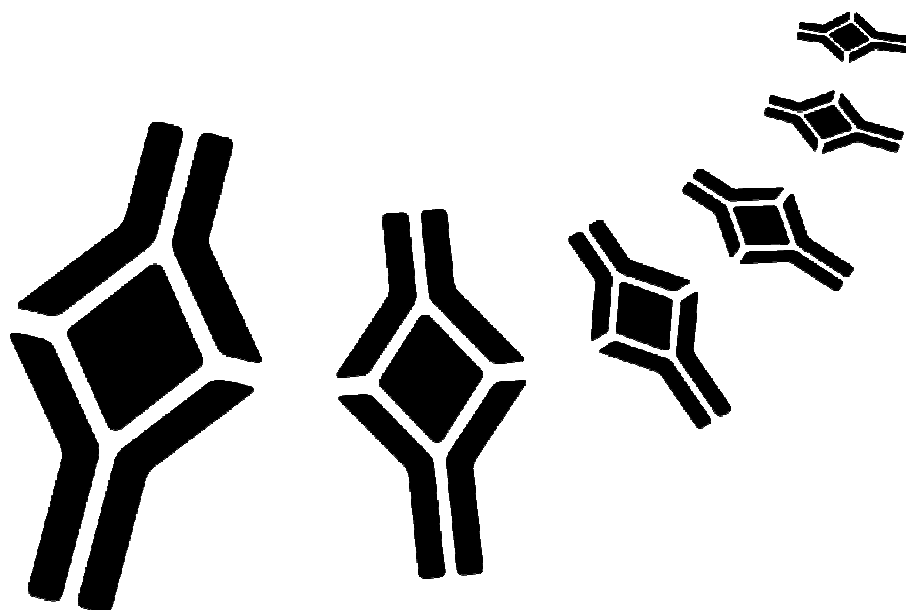


**BioVendor**

Research  
and Diagnostic Products



## HUMAN PRORENIN ELISA

Product Data Sheet

Cat. No.: RMI001R

For Research Use Only

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**»» This kit is manufactured by:  
BioVendor – Laboratorní medicína a.s.**

**»» Use only the current version of Product Data Sheet enclosed with the kit!**

## 1. INTENDED USE

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Human prorenin 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].

## 2. BACKGROUND

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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].

## 3. ASSAY PRINCIPLE

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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 450 nm. 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.

## 4. REAGENTS PROVIDED

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### ◆ Coated plate:

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

### ◆ 10X Wash Buffer:

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

### ◆ Human prorenin standard, 0 ng

2 vials of 1.5 ml lyophilized prorenin/renin depleted plasma

### ◆ Human prorenin standard, 20 ng

1 vial of 1.0 ml lyophilized high level prorenin in plasma

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

1 vial of lyophilized antibody

◆ **Horseradish peroxidase conjugated secondary antibody:**

1 vial concentrated HRP labeled antibody

◆ **TMB substrate solution:**

1 bottle of 10 ml

## 5. STORAGE AND STABILITY

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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.

## 6. REAGENTS AND EQUIPMENT REQUIRED

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- 1-channel pipettes covering 20-200 µl, 500-5000 µl and 200-1000 µl
- 12-channel pipette for 30-300 µl
- Paper towels or kimwipes
- 1.5 ml micro centrifuge tubes
- 1N H<sub>2</sub>SO<sub>4</sub>
- DI water
- Magnetic stirrer and stir-bars
- Plastic containers with lids
- TBS buffer
- Blocking buffer
- Microtiter plate spectrophotometer operable at 450 nm
- Microtiter plate shaker with uniform horizontally circular movement up to 300 rpm

## 7. WARNINGS

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**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.

## 8. PRECAUTIONS

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- **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.

## 9. PREPARATION OF REAGENTS

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

## 10. SPECIMEN PREPARATION

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Samples of human plasma and serum may be applied directly to the plate. For non plasma samples such as cell culture media, tissue extracts or urine samples, we offer the kit available with the standard curve constructed in BSA/TBS.

The assay measures human prorenin in the 0.01-10 ng/ml range.

## 11. ASSAY PROCEDURE

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

### **Preparation of Standard:**

Dilutions for the standard curve are made in prorenin/renin depleted plasma

Reconstitute standard vials as directed on each vial.

One vial at 20 ng/ml

Two vials at 0 ng/ml

Dilution table for preparation of human prorenin standards:

Prorenin concentration (ng/ml)	Dilutions
10	250 µl (0 ng/ml) + 250µl (20 ng/ml)
5	250 µl (0 ng/ml) + 250µl (10 ng/ml)
2	300 µl (0 ng/ml) + 200µl (5 ng/ml)
1	250 µl (0 ng/ml) + 250µl (2 ng/ml)
0.5	250 µl (0 ng/ml) + 250µl (1 ng/ml)
0.2	300 µl (0 ng/ml) + 200µl (0.5 ng/ml)
0.1	250 µl (0 ng/ml) + 250µl (0.2 ng/ml)
0.05	250 µl (0 ng/ml) + 250µl (0.1 ng/ml)
0.02	300 µl (0 ng/ml) + 200µl (0.05 ng/ml)
0.01	250 µl (0 ng/ml) + 250µl (0.02 ng/ml)
0	500 µl (0 ng/ml) Zero point to determine background

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

**Standard and Unknown Addition:**

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

**Primary Antibody Addition:**

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

**Secondary Antibody Addition:**

Dilute 1 µl of the conjugated secondary antibody into 10 ml BSA blocking buffer and add 100 µl to all wells. Shake plate at 300 rpm for 30 minutes. Wash wells three times with 300 µl wash buffer. Remove excess wash by gently tapping plate on paper towel or kimwipe.

### **Substrate Incubation:**

Add 100  $\mu$ l TMB substrate to all wells and shake plate for 2-10 minutes. Substrate will change from colorless to different strengths of blue. Quench reaction by adding 50  $\mu$ l of 1N H<sub>2</sub>SO<sub>4</sub> 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 and read final absorbance values at 450 nm. For best results read plate immediately.

### **Measurement:**

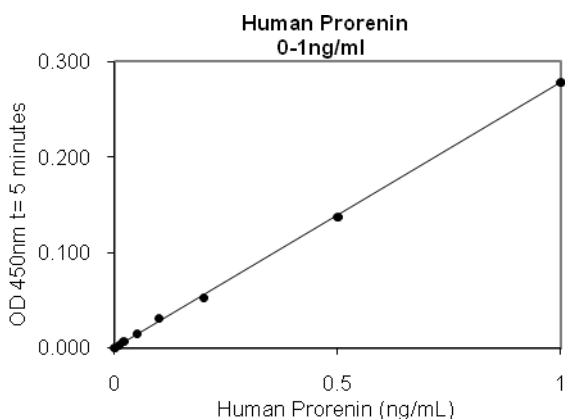
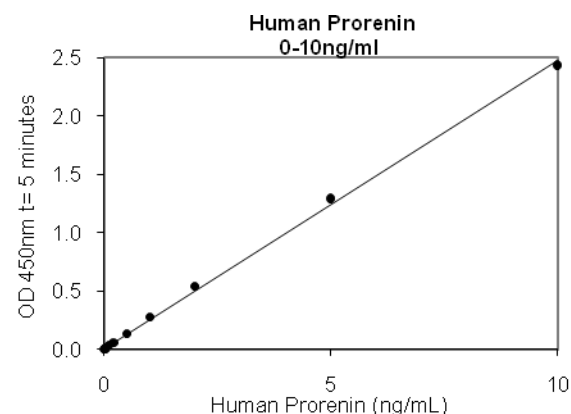
Set the absorbance at 450 nm in a microtiter plate spectrophotometer. Measure the absorbance in all wells at 450 nm. Subtract zero point from all standards and unknowns to determine corrected absorbance ( $A_{450}$ ).

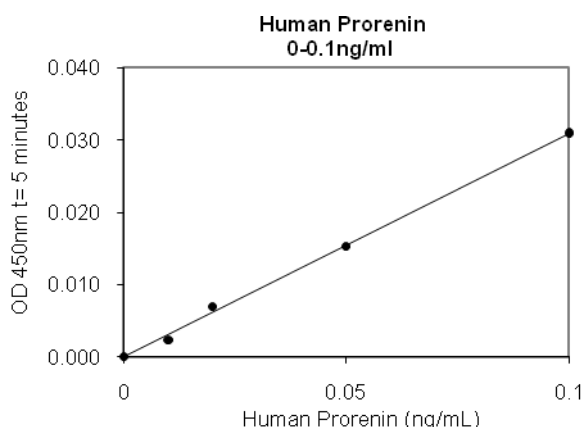
### **Assay Calibration:**

Plot  $A_{450}$  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)





## Plate Layout

### 96 Well Plate

**Standards: 22 wells**

**Samples: 74 wells**

## 12. EXPECTED VALUES

Human plasma levels of prorenin are greater in males than females and correlate positively with age and negatively with blood pressure [3]. Average plasma prorenin concentrations of 173 pg/ml (SEM=37, n=23) were found in normal control subjects with normal sodium intake by indirect methods [4]. Average serum prorenin concentrations of 109 pg/ml (SD=66, n=108) were found in normal control subjects by indirect methods [5]. Plasma and serum concentrations increase in several conditions such as pregnancy, progressive diabetes mellitus, diabetes mellitus with microvascular disease, and diabetic retinopathy [5, 6].

## 13. 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.



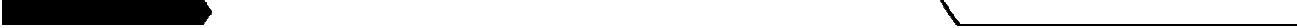
## 14. REFERENCES

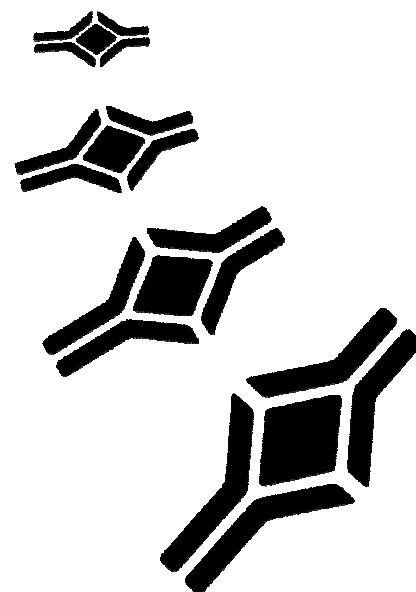
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1. Schalekamp, MADH *et al.*: Newly developed renin and prorenin assays and the clinical evaluation of renin inhibitors. *J. Hypertens.*, **26**:928-937, 2008.
2. Luetscher, JA *et al.*: Increased plasma inactive renin in diabetes mellitus. A marker 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. Toffelmire, EB *et al.*: Response of plasma prorenin and active renin to chronic and acute alterations of renin secretion in normal humans. Studies using a direct immunoradiometric assay. *J Clin Invest.*, **83**:679-687, 1989.
5. 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.
6. Schmieder, RE: The potential role of prorenin in diabetic neuropathy. *J. Hypertens.*, **25**:1323-1326, 2007.

	1	2	3	4	5	6	7	8	9	10	11	12
A	0	0.01 ng/ml	0.02 ng/ml	0.05 ng/ml	0.1 ng/ml	0.2 ng/ml	0.5 ng/ml	1 ng/ml	2 ng/ml	5 ng/ml	10 ng/ml	
B	0	0.01 ng/ml	0.02 ng/ml	0.05 ng/ml	0.1 ng/ml	0.2 ng/ml	0.5 ng/ml	1 ng/ml	2 ng/ml	5 ng/ml	10 ng/ml	
C												
D												
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NOTES





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