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Human Prorenin ELISA Kit For Non-Plasma Samples

Catalog # HPRENKT-NP

Strip well format. Reagents for up to 96 tests.

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INTENDED USE

Human prorenin assay is intended for the quantitative determination of prorenin in non-plasma samples including cell culture media, tissue extracts, or urine samples. Active renin will not be detected by this assay. Prorenin is measured directly by ELISA without pretreatment of samples or conversion to renin [1]. For quantitation of prorenin in plasma or serum samples, use the Human Prorenin ELISA Kit (cat # HPRENKT). **For research use only.**

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

- **96-well antibody coated microtiter strip plate** (removable wells 8x12) containing anti-human prorenin antibody, blocked and dried.
- **10X Wash buffer:** 1 bottle of 50ml
- **Human prorenin standard:** 1 vial lyophilized standard
- **Anti-human prorenin primary antibody:** 1 vial lyophilized monoclonal antibody
- **Anti-mouse horseradish peroxidase secondary antibody:** 1 vial concentrated HRP labeled antibody
- **TMB substrate solution:** 1 bottle of 10ml solution

STORAGE AND STABILITY

HRP conjugated secondary antibody must be stored at ≤ -70°C. Store all other kit components at 4°C upon arrival. Return any unused microplate strips to the plate pouch with desiccant. Reconstituted standards and primary may be stored at -80°C for later use. Do not freeze-thaw the standard and primary antibody more than once. Store all other unused kit components at 4°C. This kit should not be used beyond the expiration date.

OTHER REAGENTS AND SUPPLIES REQUIRED

- Microtiter plate shaker capable of 300 rpm uniform horizontally circular movement
- Manifold dispenser/aspirator or automated microplate washer
- Microplate reader capable of measuring absorbance at 450 nm
- Pipettes and Pipette tips
- Deionized or distilled water
- Polypropylene tubes for dilution of standard
- Paper towels or laboratory wipes
- 1N H_2SO_4 or 1N HCl
- Bovine Serum Albumin Fraction V (BSA)
- Tris(hydroxymethyl)aminomethane (Tris)
- Sodium Chloride (NaCl)

PRECAUTIONS

- FOR LABORATORY RESEARCH USE ONLY. NOT FOR DIAGNOSTIC USE.
- 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.
- Always pour peroxidase substrate out of the bottle into a clean test tube. Do not pipette out of the bottle as contamination could result.
- Keep plate covered except when adding reagents, washing, or reading.
- DO NOT pipette reagents by mouth and avoid contact of reagents and specimens with skin.
- 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 (BB):** 3% BSA (w/v) in TBS
- **1X Wash buffer:** Dilute 50ml of 10X wash buffer concentrate with 450ml of deionized water

SAMPLE COLLECTION

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

ASSAY PROCEDURE

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

Preparation of Standard

Reconstitute standard by adding 1ml of blocking buffer directly to the vial and agitate gently to completely dissolve contents. This will result in a 20ng/ml standard solution.

Dilution table for preparation of human prorenin standard:

Prorenin concentration (ng/ml)	Dilutions
10	500µl (BB) + 500µl (from std vial)
5	500µl (BB) + 500µl (10ng/ml)
2	600µl (BB) + 400µl (5ng/ml)
1	500µl (BB) + 500µl (2ng/ml)
0.5	500µl (BB) + 500µl (1ng/ml)
0.2	600µl (BB) + 400µl (0.5ng/ml)
0.1	500µl (BB) + 500µl (0.2ng/ml)
0.05	500µl (BB) + 500µl (0.1ng/ml)
0.02	600µl (BB) + 400µl (0.05ng/ml)
0	500µl (BB) 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 and add 100µl prorenin standards (in duplicate) and unknowns 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.

NOTE: The assay measures prorenin antigen in the 0.02-10 ng/ml range. If the unknown is thought to have high prorenin levels, dilutions may be made in blocking buffer.

Primary Antibody Addition

Reconstitute primary antibody by adding 10ml of blocking buffer directly to the 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

Briefly centrifuge vial before opening. Dilute 2µl of conjugated secondary antibody in 10ml of 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 2-10 minutes. Substrate will change from colorless to different strengths of blue. Quench reaction by adding 50µl of 1N H_2SO_4 or HCl 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 by gently shaking the plate.

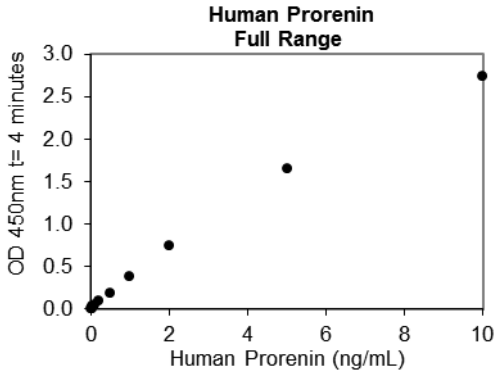
Measurement

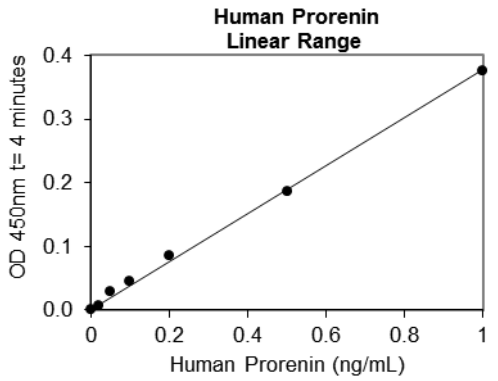
Set the absorbance at 450nm in a microtiter plate spectrophotometer. Measure the absorbance in all wells at 450nm. Subtract zero point from all standards and unknowns to determine corrected absorbance (A_{450}).

Calculation of Results

Plot A_{450} against the amount of prorenin in the standards. Fit a straight line through the linear points of the standard curve using a linear fit procedure if unknowns appear on the linear portion of the standard curve. Alternatively, create a standard curve by analyzing the data using a software program capable of generating a four parameter logistic (4PL) curve fit. The amount of prorenin in the unknowns can be determined from this curve. If samples have been diluted, the calculated concentration must be multiplied by the dilution factor.

A typical standard curve (EXAMPLE ONLY):





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].

PERFORMANCE CHARACTERISTICS

Sensitivity: The minimum detectable dose (MDD) was determined by adding two standard deviations to the mean optical density value of twenty zero standard replicates (range OD_{450}: 0.063-0.071) and calculating the corresponding concentration. The MDD was 0.016 ng/ml.

Intra-assay Precision: Three samples of known concentration were tested twenty times on one plate to assess intra-assay precision.

Sample	1	2	3
n	20	20	20
Mean (ng/ml)	0.126	0.523	5.12
Standard Deviation	0.009	0.028	0.243
CV (%)	7.28	4.88	4.74

Inter-assay Precision: Three samples of known concentration were tested in ten independent assays to assess inter-assay precision.

Sample	1	2	3
n	10	10	10
Mean (ng/ml)	0.165	0.532	5.03
Standard Deviation	0.016	0.031	0.203
CV (%)	9.70	5.85	4.04

Recovery: The recovery of antigen spiked to levels throughout the range of the assay in culture media was evaluated.

Sample	1	2	3	4
n	4	4	4	4
Mean (ng/ml)	0.230	0.796	2.014	5.67
Average % Recovery	115	100	107	113
Range	104-132%	98-104%	101-111%	110-117%

Linearity: To assess the linearity of the assay, samples of culture media spiked with high concentrations of antigen were serially diluted to produce samples with values within the dynamic range of the assay.

Sample	1:2	1:4	1:8	1:16
n	4	4	4	4
Average % of Expected	99	93	97	104
Range	96-102%	91-95%	93-102%	101-107%

Specificity: This assay recognizes recombinant and natural human prorenin. The factors listed below were prepared at 10 ng/ml in blocking buffer and assayed for cross-reactivity. No significant cross-reactivity was observed.

Recombinant human renin
Recombinant mouse prorenin
Recombinant rat prorenin

Sample Values: Samples were evaluated for the presence of the antigen.

Sample Type	Mean (ng/mL)
Urine, Fresh	0.59
Urine, Frozen	0.35
Milk, Frozen	1.4

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 of or contact with the above product.

REFERENCES

1. Schalekamp MADH, et al.: J Hypertens., 2008, 26:928-937.
2. Luetscher JA, et al.: N Engl J Med. 1985, 312:1412-1417.
3. Danser AH, et al.: J Hypertens. 1988, 16:853-862.
4. Toffelmire EB, et al.: J Clin Invest. 1989, 83:679-687.
5. Yokota H, et al.: Br J Ophthalmol. 2005, 89:871-873.
6. Schmieder RE: J Hypertens. 2007, 25:1323-1326.

Example of ELISA Plate Layout
96 Well Plate: 20 Standard wells, 76 Sample wells

	1	2	3	4	5	6	7	8	9	10	11	12
A	0	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	0.02 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												
E												
F												
G												
H												