YK260 Human Prorenin (open form)

ELISA Product Instructions

FOR LABORATORY USE ONLY

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Contents

– Please read all the package insert carefully before beginning the assay –

YK260 Prorenin (open form) ELISA Kit

I. Introduction

Prorenin is the inactive proenzyme of renin, which is produced by cleavage of a 23 amino acids long signal peptide from preprorenin[(1)]. Prorenin can be either excreted immediately or transformed into mature renin by the cleavage of 43 amino acids long prosegment from the N-terminal end. In addition of juxtaglomerular sells, several extrarenal sites have been identified that produce prorenin, whereas renin production is mostly limited to the kidney. Prorenin exists in an equilibrium conformations as two forms with the prosegment of N-terminal closed or opened. By binding to pro(renin) receptor, closed, inactive form of prorenin transformed to open, active form of prorenin which acts like as renin to produce angiotensin I without proteolytic cleavage of the prosegment. Opening the prosegment can be achieved by exposure to low pH (pH=3.0) or cold temperature reversibly or by renin inhibitor such as aliskiren irreversibly. The aliskiren induced open form of prorenin has no any enzymatic activity to angiotensinogen. High levels of prorenin were observed in diabetes complicated by nephropathy and retinopathy and the occurrence of microalbuminuria[(2)]. Some research showed that when renal mesangial cells were incubated with high concentration of prorenin, the angiotensin I generation ability was acquired addition with TGF-b1, PAI-1, fibronectin mRNA expression were significantly induced[(3)]. The development of insulin resistance was associated with nonproteolytic activation of prorenin as well as local angiotensin II generation in skeletal muscle and adipose tissues of obese rats[(4)]. So the investigation for nonproteolytic activated prorenin (i.e. open form prorenin) level seems to be very important to elucidate the possible pathologic role of prorenin in diabetes and hypertension diseases.

But to date, there is no any quantitative product specific for open form prorenin available. The present available commercial assay kit for direct determination of prorenin cannot recognize well for the open form prorenin. In other hand, in hypertension patients administered with aliskiren, the equilibrium of close/open form of prorenin may be distinctly altered predictably. So the prorenin level measured by present assay kit can not represent the real level of prorenin in such cases. From this point, our new developed ELISA assay kit can direct measure open form prorenin specifically without pretreatment of samples or converting to renin by enzymatic proteolysis. It is specially suitable for the necessary when evaluation of the level of open form prorenin is important such as in patients when administered aliskiren for treatment, in addition to diabetes research etc..

YK260 Human Prorenin (open form) Elisa Kit	**Contents**
▼ The kit quantitative range (linear): 25-6000pg/mL.	1) Antibody Coated Plate
▼ The assay running time: 5.5 h.	2) Standard
▼ Maximum measurable samples: 41 in duplicate	3) HRP-Labeled Antibody
▼ Test sample (size): human plasma (50μL)	4) Buffer Solution A
▼ The kit can be used dividedly in strips.	5) Buffer Solution B
▼ Intra-assay %CV: 1.3-3.1	6) Concentrated Wash Solution
▼ Inter-assay %CV: 2.7-4.2	7) TMB Substrate
	8) Reaction Stopping Solution
	9) Adhesive Foil

II. Characteristics

This ELISA kit is designed for specifically quantitative determination of Prorenin (open form) in human plasma. It has various advantages, such as no extraction procedure of samples, short assay time, practically no influences of other body fluids or physiological active substances coexisting in samples assayed.

< Specificity **>**

This ELISA kit specially recognizes human prorenin (open form). The cross-reaction of human prorenin (closed form) and human renin is not observed with this assay system. But about 2% reactivity with native recombinant human prorenin will be observed at room condition for the existence of an equilibrium of close/open prorenin in buffer solution. About 4.6% cross-reactivity was observed with human preprorenin (21-64).

< Assay Principle **>**

This ELISA kit for determination of prorenin (open form) is based on an enzyme linked immuno-sorbent assay using combination of monoclonal antibody coated to plate which specific capture the prosegment of prorenin (open form) and rabbit anti-prorenin (open form)- horseradish peroxidase (HRP) conjugate (HRP-labeled anti-prorenin (open form)) system. I.e. Prorenin (open form) standard or samples are added to plate; after incubation and plate washing, captured prorenin (open form) is further reacted with HRP labeled rabbit anti-prorenin (open form) second antibody. After incubation and plate washing, HRP enzyme activity is determined by 3,3',5,5'-tetramethylbenzidine (TMB) and the concentration of Prorenin (open form) is then calculated.

III. Composition

	Component	Form	Quantity	Main Ingredient
1	Antibody Coated Plate	Microtiter plate	1 plate (96 wells)	Monoclonal antibody to preprorenin (21-64) coated plate
2	Standard	Lyophilized powder	1 vial (18ng)	Recombinant prorenin (open form)
3	HRP-Labeled antibody	Liquid	1 vial (12mL)	HRP conjugated rabbit anti- Prorenin (open form) antibody
4	Buffer Solution A	Liquid	1 bottle (7 mL)	A special designed phosphate buffer
5	Buffer Solution B	Liquid	1 bottle (5 mL)	Aliskiren containing phosphate buffer
6	Concentrated Wash Solution	Liquid	1 bottle (25 mL)	Concentrated saline
7	TMB Substrate	Liquid	1 bottle (12 mL)	3,3',5,5'-Tetramethylbenzidine (TMB)
8	Reaction Stopping Solution	Liquid	1 bottle (12 mL)	1M Sulfuric acid
9	Adhesive Foil		1 sheet	

IV. Method

< Equipment required **>**

1. Photometer for microtiter plate (plate reader), which can read extinction 2.5 at 450 nm
2. Washing device for microtiter plate and dispenser with aspiration system
3. Micropipettes for volumes between 10 μL –1000 μL
4. Multi-channel pipettes for 8 or 12 wells and the tips
5. Polypropyrene test tubes for preparation of standard
6. A microplate shaker
7. Graduated cylinder (500 mL or 1,000 mL)
8. Deionized distilled water

< Preparatory work **>**

1. Preparation of Prorenin (open form) standard solution: Reconstitute lyophilized **Standard** with 0.5mL of **Buffer Solution B**, which affords 36000pg/mL stock solution. The reconstituted Prorenin (open form) stock solution (0.1mL) is diluted with 0.5mL of **Buffer Solution B** that yields 6000pg/mL standard solution. Then 0.2mL of 6000pg/mL standard solution is diluted with 0.4mL of **Buffer Solution B** that yields 2000pg/mL standard solution. Repeat the same dilution to make standard solution of 667, 222, 74 pg/mL, and 25pg/mL respectively. **Buffer Solution B** is used as 0 ng/mL.
2. Dilution of Wash Solution Concentrated: Dilute one bottle of Wash Solution Concentrated (25 mL) to 500 mL with deionized distilled water.
3. Other reagents are ready for use.

< Assay procedure **>**

1. Before starting assay, bring all the reagents, except test samples, to room temperature (22-25°C).
2. Pipet 50 μL of **Buffer Solution A** into each well.
3. Pipet 50 μL of standard solutions (0, 25, 74, 222, 667, 2000, and 6000pg/mL) or samples into appropriate wells.
4. Cover the plate with adhesive foil and incubate it on a shaker at room temperature for 4 hours.
5. After incubation, take off the adhesive foil, aspirate or decant the solutions in the wells. Add 350 μL of diluted wash solution to each well and keep it for about 30 seconds, and then aspirate or decant the wash solution in the wells. Repeat this wash process 4 times (total 5 times). Finally, invert the plate and tap it onto an absorbent surface, such as paper toweling, to ensure blotting free of most residual wash solution.
6. Add 100μL of **HRP-Labeled Antibody** into each well
7. Cover the plate with adhesive foil and incubate it on a shaker at room temperature for 1 hour.
8. After incubation, repeat the step 5 for washing.
9. Add 100 μL of **TMB Substrate** into each well.

10. Cover the plate with adhesive foil and incubate it on a shaker at room temperature for 30 minutes under the light proof condition.
11. Add 100 µL of **Reaction Stopping Solution** into each well to stop color reaction.
12. Read the absorbance of the wells at 450 nm with 620nm as reference wave.
13. After draw the blank (0 pg/mL) for each well, plot the absorbance against the amount of prorenin (open form) in standards. The prorenin (open form) concentration of unknowns can be determined by from this curve. A linear curve can be observed between 2000pg/mL to 0pg/mL.

V. Notes

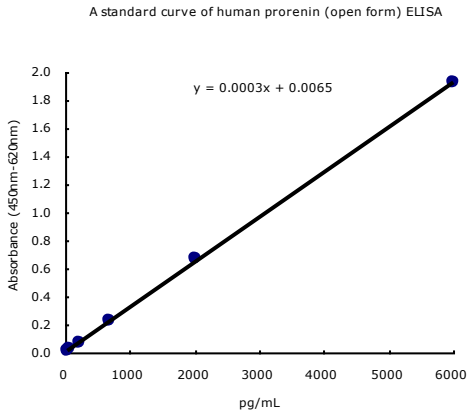
1. It is recommended that EDTA-2Na plasma samples should be used as soon as possible after collection. Please note that because there is an equilibrium of close/open form of prorenin and the conversion of closed form to open form is temperature dependent, so the assay should be carried out without refrigerator or freezing keeping after sample collecting. If the sample is tested later, they should be aliquoted and frozen below −30°C (for long term storage, in a −80°C deep freezer). But the proportion of open form prorenin in the sample will rise to some content during the storage. Avoid repeated freezing and thawing of samples.
2. Prorenin (open form) standard solution should be prepared immediately before use. If the kit used dividedly, the rests of the reconstituted Prorenin (open form) standard solution (18000 pg/mL), **HRP-labeled Antibody** and other reagents except wash solution and stopping solution should be stored at 4°C and used within 2 weeks. Diluted standard solutions should not be reused for another assay.
3. Incomplete washing of the microplate will interfere with assay precision. If a microplate washer is not available, completely aspirate the solutions in the wells of assay plate to be removed or decant them by inverting the plate and tapping it onto absorbent tissue in each wash cycle. Ensure that there is no residual wash solution in the wells after final wash.
4. As pipetting operations may affect precision of the assay, pipet standard solutions or samples precisely into the wells of assay plate. In addition, use clean test tubes or vessels in assay and a new tip for each standard diluting process and for each sample or standard solution pipetting to avoid cross contamination.
5. Perform all the determination in duplicate.
6. To quantitate accurately, always run a standard curve for each assay.
7. Color reaction should be carried out under the light proof condition.
8. Read optical absorbance of reaction solution in wells as soon as possible after stopping the color reaction.
9. Protect the reagents from strong light (e.g. direct sunlight) during storage and assay.
10. Satisfactory performance of the assay will be guaranteed only when reagents are used from combination pack with identical lot number.

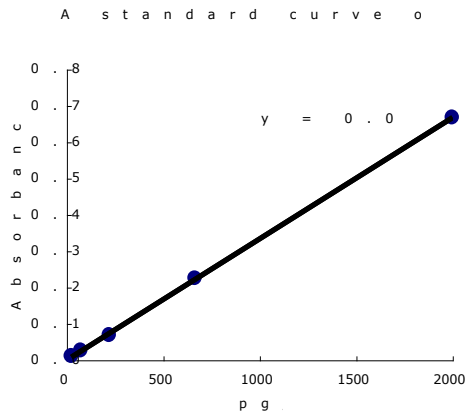
VI. Performance Characteristics

< Assay range **>**

25 – 6000 pg/mL

<A typical standard curve>





< Precision and reproducibility **>**

Intra-assay variation	Measured (pg/mL)	%CV
QC sample 1	224±7	3.1
QC sample 2	364±10	2.8
QC sample 3	1198±15	1.3

(mean±SD, n=10)

Inter-assay variation	Measured (pg/mL)	%CV
QC sample 4	277±12	4.2
QC sample 5	679±28	4.1
QC sample 6	2366±64	2.7

(mean±SD, n=5)

< Analytical recovery **>**

Human plasma	Prorenin (open form) added (pg/mL)	Observed (pg/mL)	Expected (pg/mL)	Recovery (%)
No.1	0	137		
	66	201	203	99.0
	595	710	732	97.0
	1784	1864	1921	97.0
No.2	0	85		
	66	164	151	108.6
	595	646	680	95.0
	1784	1881	1869	100.6
No.3	0	87		
	66	180	153	117.6
	595	766	682	112.3
	1784	2004	1871	107.1

<Dilution test**>**

Human plasma	Dilution ratio (1X)	Observed (pg/mL)	Expected (pg/mL)	% of expected
No.1	1	204		
	2	86	102	84.3
	4	61	51	119.6
No.2	1	172		
	2	79	86	91.9
	4	58	43	134.9
No.3	1	185		
	2	93	93	100
	4	53	46	115.2

<Specificity**>**

Human prorenin (open form) 100%, human preprorenin (21-64) 4.6%, human prorenin (close form) 0%, human renin 0%.

VII. Stability and Storage

< Storage **>** Store all the components in the kit at 2°C - 8°C.

< Shelf Life **>** The kit is stable under the storage condition for 6 months temporary from the date of manufacture.
The expiry date is stated on the label of package.

< Package **>** For 96 tests per one kit including standards.

VIII. References

1. Imai T, Miyazaki H et al: **Cloning and sequence analysis of cDNA for human renin precursor.** *Proc. Natl. Acad. Sci.* 80:7405-7409, 1983
2. Luetscher JA. Kreamer FB et al: **Increased plasma inactive renin in diabetes mellitus. A marker of microvascular complications.** *N Engl J Med*. 312:1412-1417, 1985
3. Zhang J, Wu J et al: **Receptor-mediated nonproteolytic activation of prorenin and induction of TGF-β_1 and PAI-1 expression in renal mesangial cells.** *Renal Physiol.* 303:no. 1 F11-F20, 2012
4. Rafiq K, Mori H et al: **(Pro)renin receptor and insulin resistance: Possible roles of angiotensin II-dependent and -independent pathways.** *Mol. Cell Endocrinol*., 2012, Jun 7 Epub

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