PRODUCT INFORMATION & MANUAL

Human Renin Valukine™ ELISA

Catalog Number: VAL244

For the quantitative determination of natural and recombinant human mature and pro-Renin concentrations

For research use only.
Not for diagnostic or therapeutic procedures.

Bio-Techne China Co. Ltd
P: +86 (21) 52380373 P: 8009881270 F: +86 (21) 52381001
info.cn@bio-techne.com

Please refer to the kit label for expiry date.
Novus kits are guaranteed for 3 months from date of receipt

Version 202411.1

TABLE OF CONTENTS

I. BACKGROUND

Renin is an N-glycosylated member of the aspartyl proteinase family and plays a critical role in the activation of the Renin-Angiotensin-Aldosterone System (RAAS). RAAS activity is involved in the regulation of blood pressure, sodium balance, inflammation, immunity, intracellular redox balance, glucose homeostasis, and the function of adipocytes and hematopoietic progenitor cells (1-5). Renin differs from other members of this family by its optimal activity near neutral pH instead of in the pH 2.0 to 3.4 range. This more neutral pH optimum enables Renin to be functional in the plasma. Renin is highly specific for the cleavage of Serpin A8/ Angiotensinogen, its only known physiological substrate. Renin cleavage of Angiotensinogen releases the decapeptide Angiotensin I, which is further cleaved by Angiotensin Converting Enzyme (ACE) and ACE-2 into the bioactive peptides Angiotensin II, Ang1-9, and Ang1-7. Angiotensin II is a potent effector of RAAS activity.

The 47 kDa human Prorenin, consisting of a 43 amino acid (aa) propeptide and a 340 aa active enzyme, is secreted as an inactive enzyme (6-8). It becomes activated in the circulation by proteolytic removal of the propeptide. Alternatively, the propeptide can be removed intracellularly, followed by the secretion of active 44 kDa Renin (7, 9, 10). Mature Renin can be further cleaved into 22 kDa and 18 kDa fragments which exhibit enzymatic activity as long as they remain associated (8). Human Prorenin shares 70% and 66% aa sequence identity with mouse and rat Prorenin, respectively. Active human Renin shares 71% and 67% aa sequence identity with mouse and rat Renin, respectively. Renin is produced by juxtaglomerular cells in the kidney in response to renal sympathetic nerve activity, low sodium levels, low blood pressure, prostanoids, and a variety of hormones (1, 11, 12). Prorenin is also expressed at several extra-renal sites including the ovary and pancreas. The generation of Angiotensin II is important for follicular development in the ovary and for regulating pancreatic glucose metabolism and insulin resistance (3, 13, 14).

Circulating Prorenin and Renin bind to the Renin Receptor (Renin R), also known as V-ATPase accessory protein 2 (15-19). Renin R is expressed on renal mesangial cells, coronary and renal artery subendothelium, vascular smooth muscle cells, placental vasculature, and syncytiotrophoblasts (15). Renin R binding of Prorenin can induce Renin enzymatic activity without proteolytic removal of the propeptide (15, 16). Renin R is a transmembrane protein, but a 28 kDa soluble form can be secreted and retains the ability to bind Renin (20). Circulating Prorenin can trigger mesangial cell proliferation and can be internalized by cardiac myocytes (21, 22).

II. OVERVIEW

A. PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. An antibody specific for human Renin has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any human Renin present is bound by the immobilized antibody. After washing away any unbound substances, an enzyme-linked antibody specific for human Renin is added to the wells. Following a wash to remove any unbound antibody-enzyme reagent, TMB substrate (Chromogenic agent) is added to the wells and color develops in proportion to the amount of human Renin bound in the initial step. The color development is stopped, and the intensity of the color is measured.

B. LIMITATIONS OF THE PROCEDURE

- **FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.**
- This kit is suitable for cell culture supernates, human serum, human plasma and human urine.
- The kit should not be used beyond the expiration date on the kit label.
- Do not mix or substitute reagents with those from other lots or sources.
- If samples generate values higher than the highest standard, dilute the samples with Calibrator Diluent (1×) and repeat the assay.
- Any variation in operator, pipetting technique, washing technique, incubation time or temperature, and kit age can cause variation in binding.

III. ADVANTAGES

A. PRECISION

Intra-assay Precision (Precision within an assay)

Three samples were tested twenty times on one plate to assess intra-assay precision.

Inter-assay Precision (Precision between assays)

Three samples were tested in twenty separate assays to assess inter-assay precision.

	Intra-assay Precision			**Inter-assay Precision**		
Sample	1	2	3	1	2	3
Mean (pg/mL)	201	599	1193	191	566	1153
Standard Deviation	10.6	14.2	19.9	10.5	27.1	46.4
CV%	5.3	2.4	1.7	5.5	4.8	4.0

B. RECOVERY

The recovery of human Renin spiked to levels throughout the range of the assay in various matrices was evaluated.

Sample Type	**Average % Recovery**	**Range (%)**
Cell culture media (n=8)	100	93-106
Human Serum* (n=4)	101	90-109
Human EDTA plasma* (n=4)	101	95-107
Human Heparin plasma* (n=4)	105	99-114
Human Urine (n=4)	93	80-106

* Samples were diluted prior to assay.

C. SENSITIVITY

Twenty assays were evaluated and the minimum detectable dose (MDD) of human Renin ranged from 0.769-14.8 pg/mL. The mean MDD was 4.43 pg/mL.

The MDD was determined by adding two standard deviations to the mean optical density value of twenty zero standard replicates and calculating the corresponding concentration.

D. CALIBRATION

This immunoassay is calibrated against a highly purified NS0-expressed recombinant human pro-Renin produced at R&D Systems. The NIBSC/WHO International Standard for Renin (68/356), which was intended as a potency standard, was evaluated in this kit. The NIBSC/WHO standard is a purified extract of human kidney.

The dose response curve of the International Standard (68/356) parallels the Valukine standard curve. To convert sample values obtained with the Valukine Human Renin kit to approximate NIBSC 68/356 units, use the equation below.

NIBSC (68/356) approximate value (IU/mL) =1.58 × 10^{-7} × Valukine Human Renin value (pg/mL)

E. LINEARITY

To assess the linearity of the assay, different samples were containing or spiked with high concentrations of human Renin and diluted with Calibrator Diluent (1×) to produce samples with values within the dynamic range of the assay.

Dilution		Cell culture media (n=8)	Human Serum* (n=4)	Human EDTA plasma* (n=4)	Human Heparin plasma* (n=4)	Human Urine (n=4)
1:2	Average % of Expected	98	101	99	101	100
	Range (%)	95-102	99-103	96-102	100-102	96-103
1:4	Average % of Expected	99	106	100	102	101
	Range (%)	96-102	104-107	96-104	99-107	96-106
1:8	Average % of Expected	102	108	103	102	102
	Range (%)	94-108	103-114	95-108	94-111	96-107
1:16	Average % of Expected	98	105	108	106	107
	Range (%)	88-108	100-109	96-119	97-112	98-115

*Samples were diluted prior to assay.

F. SAMPLE VALUES

Human Serum/Plasma/Urine - Samples from apparently healthy volunteers were evaluated for the presence of human Renin in this assay. No medical histories were available for the donors used in this study.

Sample Type	Mean (pg/mL)	Range (pg/mL)	Standard Deviation (pg/mL)
Human Serum (n=35)	776	232-1892	374
Human EDTA plasma (n=35)	726	201-1852	365
Human Heparin plasma (n=35)	680	179-1760	349

Sample Type	Mean of Detectable (pg/mL)	% Detectable	Range (pg/mL)
Human Urine (n=12)	60.4	42	ND-96.7

ND=Non-detectable

Cell Culture Supernates - CCD-1070Sk human foreskin fibroblasts were cultured in MEM supplemented with 10% fetal bovine serum and 1 mM sodium pyruvate and grown for 8 days until confluent. An aliquot of the cell culture supernate was removed, assayed for human Renin, and measured 197 pg/mL.

G. SPECIFICITY

This assay recognizes natural and recombinant human Renin. This assay recognizes both the mature and pro-versions of recombinant human Renin.

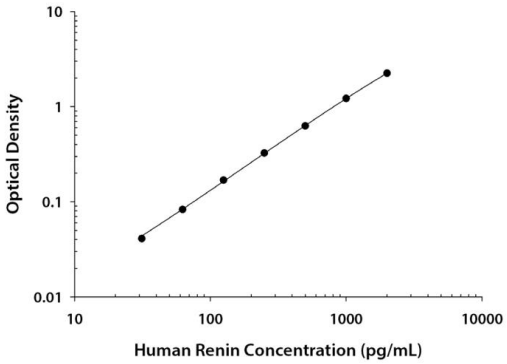
The factors listed below were prepared at 50 ng/mL in Calibrator Diluent (1×) and assayed for cross-reactivity. Preparations of the following factors at 50 ng/mL in a mid-range recombinant human Renin control were assayed for interference. No significant cross-reactivity or interference was observed.

Recombinant human:	**Recombinant mouse:**
ACE/CD143	ACE/CD143
ACE-2	Renin
Cathepsin B	Serpin A8/Angiotensinogen
Cathepsin D	**Recombinant rat:**
Cathepsin E	Renin
Renin R	
Serpin A8/Angiotensinogen	

IV. EXPERIMENT

EXAMPLE STANDARD

This standard curve is provided for demonstration only. A standard curve should be generated for each set of samples assayed.



(pg/mL)	O.D.	Average	Corrected
0	0.011 0.013	0.012	—
31.3	0.051 0.055	0.053	0.041
62.5	0.095 0.095	0.095	0.083
125	0.179 0.183	0.181	0.169
250	0.333 0.341	0.337	0.325
500	0.638 0.638	0.638	0.626
1000	1.220 1.241	1.231	1.219
2000	2.248 2.264	2.256	2.244

V. KIT COMPONENTS AND STORAGE

A. MATERIALS PROVIDED

Parts	Description	Size
Human Renin Microplate	96 well polystyrene microplate (12 strips of 8 wells) coated with an antibody against human Renin	1 plate
Human Renin Conjugate	Solution of antibody against human Renin conjugated to horseradish peroxidase	1 vial
Human Renin Standard	Recombinant human pro-Renin in a buffered protein base; lyophilized. Refer to the vial label for reconstitution volume	1 vial
Assay Diluent RD1S	A buffered protein base	1 vial
Calibrator Diluent Concentrate (5×)/ RD5P	A 5× concentrated buffered protein base used to dilute standard and samples	1 vial
Wash Buffer Concentrate (25×)	A 25× concentrated solution of buffered surfactant	1 vial
TMB Substrate	TMB ELISA Substrate Solution/TMB Substrate Solution	2 vials
Stop Solution	2 N sulfuric acid	1 vial
Plate Sealers	Adhesive strip	3 strips

B. STORAGE

<table>
<tr><td>Unopened Kit</td><td colspan="2">Store at 2-8°C. Do not use past kit expiration date.</td></tr>
<tr><td rowspan="6">Opened/
Reconstituted
Reagents</td><td>Wash Buffer (1×)</td><td rowspan="4">May be stored for up to 1 month at 2-8°C.*</td></tr>
<tr><td>Stop Solution</td></tr>
<tr><td>Conjugate</td></tr>
<tr><td>TMB Substrate</td></tr>
<tr><td>Standard</td><td>May be stored for up to 1 month at 2-8 °C.*</td></tr>
<tr><td>Assay Diluent
RD1S</td><td>May be stored for up to 1 month at 2-8 °C.*</td></tr>
<tr><td>Calibrator Diluent
Concentrate (5×)/
RD5P</td><td>May be stored for up to 1 month at 2-8 °C.*
Use and discard diluted Calibrator Diluent (1×). Prepare fresh for each assay.</td></tr>
<tr><td>Microplate Wells</td><td>Return unused wells to the foil pouch containing the desiccant pack, reseal along entire edge of zip-seal. May be stored for up to 1 month at 2-8°C.*</td></tr>
</table>

* Provided this is within the expiration date of the kit.

C. OTHER SUPPLIES REQUIRED

- Microplate reader capable of measuring absorbance at 450 nm, with the correction wavelength set at 540 nm or 570 nm.
- Pipettes and pipette tips.
- Deionized or distilled water.
- Squirt bottle, manifold dispenser, or automated microplate washer.
- 100 mL and 500 mL graduated cylinder.
- Horizontal orbital shaker (0.12” orbit) capable of maintaining a speed of 500±50 rpm.
- Test tubes for dilution of standards and samples.

D. PRECAUTION

- Some components in this kit contain a preservative which may cause an allergic skin reaction. Avoid breathing mist.
- The Stop Solution provided with this kit is an acid solution. Wear eye, hand, face, and clothing protection when using this material.

VI. PREPARATION

A. SAMPLE COLLECTION AND STORAGE

The sample collection and storage conditions listed below are intended as general guidelines. Sample stability has not been evaluated.

Cell Culture Supernates - Remove particulates by centrifugation and assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles. Samples may require dilution with Calibrator Diluent (1×).

Serum - Use a serum separator tube (SST) and allow samples to clot for 30 minutes at room temperature before centrifugation for 15 minutes at 1000 × g. Remove serum and assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles. Samples may require dilution with Calibrator Diluent (1×).

Plasma - Collect plasma using heparin or EDTA as an anticoagulant. Centrifuge for 15 minutes at 1000 × g within 30 minutes of collection. Assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles. Samples may require dilution with Calibrator Diluent (1×).

Note: *Citrate plasma has not been validated for use in this assay.*

Hemolyzed samples are not recommended for use in this assay.

Urine - Aseptically collect the first urine of the day (mid-stream), voided directly into a sterile container. Centrifuge to remove particulate matter, and assay immediately or aliquot and store at ≤ -20 °C. Avoid repeated freeze-thaw cycles. Samples may require dilution with Calibrator Diluent (1×).

B. SAMPLE PREPARATION

Human serum and plasma samples recommend a 2-fold dilution. A suggested 2-fold dilution is 75 µL of sample + 75 µL of Calibrator Diluent (1×).

C. REAGENT PREPARATION

Bring all reagents to room temperature before use.

Wash Buffer (1×) - If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. Dilute 20 mL of Wash Buffer Concentrate (25×) into deionized or distilled water to prepare 500 mL of Wash Buffer (1×).

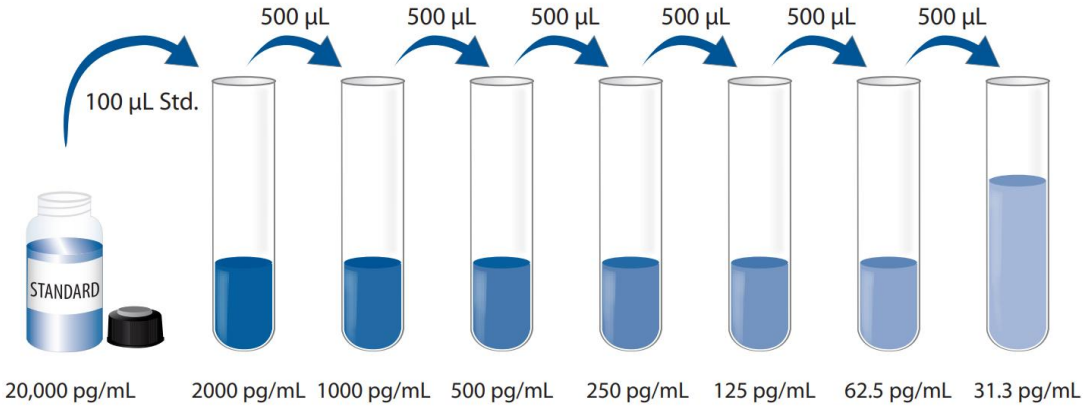
Calibrator Diluent (1×) - Use deionized or distilled water to prepare Calibrator Diluent (1×).

Human Renin Standard - Refer to the vial label for the reconstitution volume*. Reconstitute the Human Renin Standard with deionized or distilled water. This reconstitution produces a stock solution of 20000 pg/mL. Allow the standard to sit for

a minimum of 15 minutes with gentle agitation prior to making dilutions.

*If you have any question, please seek help from our Technical Support.

Pipette 900 µL of Calibrator Diluent (1×) into the 2000 pg/mL tube. Pipette 500 µL into the remaining tubes. Use the stock solution to produce a dilution series (below). Mix each tube thoroughly before the next transfer. The 2000 pg/mL standard serves as the high standard. Calibrator Diluent (1×) serves as the zero standard (0 pg/mL).



D. TECHNICAL HINTS

- When mixing or reconstituting protein solutions, always avoid foaming.
- To avoid cross-contamination, change pipette tips between additions of each standard level, between sample additions, and between reagent additions. Also, use separate reservoirs for each reagent.
- It is recommended that the samples be pipetted within 15 minutes.
- To ensure accurate results, proper adhesion of plate sealers during incubation steps is necessary.
- TMB Substrate should remain colorless until added to the plate. Keep TMB Substrate protected from light. TMB Substrate should change from colorless to gradations of blue.
- Stop Solution should be added to the plate in the same order as the TMB Substrate. The color developed in the wells will turn from blue to yellow upon addition of the Stop Solution. Wells that are green in color indicate that the Stop Solution has not mixed thoroughly with the TMB Substrate.

VII.ASSAY PROCEDURE

Bring all other reagents and samples to room temperature before use. It is recommended that all standards and samples be assayed in duplicate.

1. Prepare all reagents and working standards as directed in the previous sections.
2. Remove excess microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, and reseal.
3. Add 100 µL of Assay Diluent RD1S to each well.
4. Add 50 µL of standard and prepared sample per well. Cover with the adhesive strip provided. **Incubate for 2 hours at room temperature on a horizontal orbital shaker (0.12" orbit) set at 500±50 rpm.** A plate layout is provided for a record of standards and samples assayed.
5. Aspirate each well and wash, repeating the process three times for a total of four washes. Wash by filling each well with Wash Buffer (400 µL) using a squirt bottle, manifold dispenser, or autowasher. Complete removal of liquid at each step is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against clean paper towels.
6. Add 200 µL of Human Renin Conjugate to each well. Cover with a new adhesive strip. **Incubate for 2 hours at room temperature on a horizontal orbital shaker (0.12" orbit) set at 500±50 rpm.**
7. Repeat the aspiration/wash as in step 5.
8. Add 200 µL of TMB Substrate to each well. **Incubate for 30 minutes at room temperature. Protect from light**.
9. Add 50 µL of Stop Solution to each well. Gently tap the plate to ensure thorough mixing.
10. Determine the optical density of each well within 10 minutes, using a microplate reader set to 450 nm. If wavelength correction is available, set to 540 nm or 570 nm. If wavelength correction is not available, subtract readings at 540 nm or 570 nm from the readings at 450 nm. This subtraction will correct for optical imperfections in the plate. Readings made directly at 450 nm without correction may be higher and less accurate.

11. CALCULATION OF RESULTS

Average the duplicate readings for each standard and sample and subtract the average zero standard optical density (O.D.).

Create a standard curve by reducing the data using computer software capable of generating a four-parameter logistic (4-PL) curve-fit. As an alternative, construct a standard curve by plotting the mean absorbance for each standard on the y-axis against the concentration on the x-axis and draw a best fit curve through the points on the graph. The data may be linearized by plotting the log of the human Renin concentrations versus the log of the O.D. and the best fit line can be determined by regression analysis. This procedure will produce an adequate but less precise fit of the data.

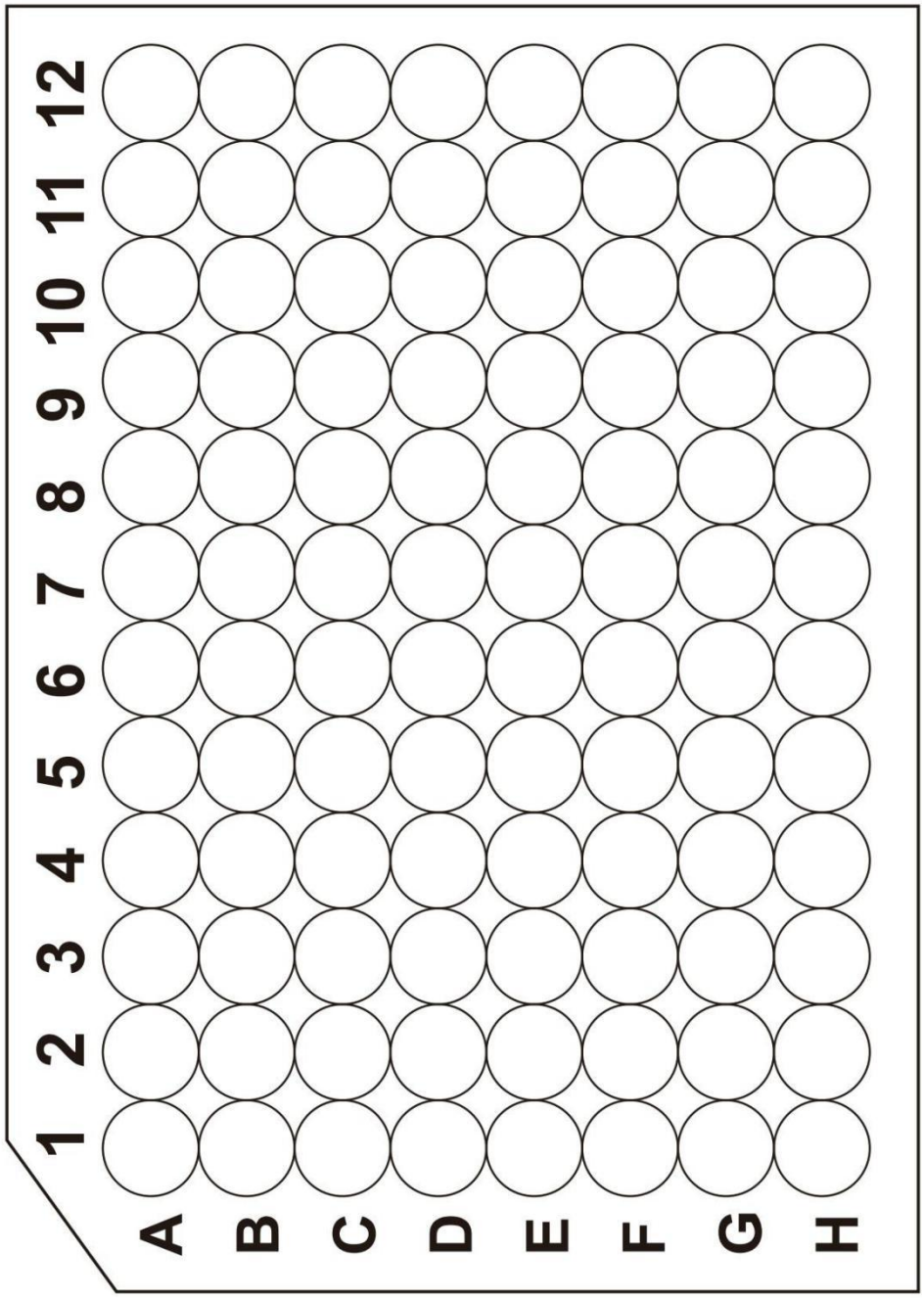
If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.

VIII. REFERENCES

1. Castrop, H. *et al*. (2010) Physiol. Rev. 90:607.
2. Capettini, L.S.A. *et al*. (2012) Curr. Pharmaceut. Des. 18:963.
3. Luther, J.M. and N.J. Brown (2011) Trends Pharmacol. Sci. 32:734.
4. Jing, F. *et al.* (2012) Mol. Cell. Endocrinol. Epub. PMID 22465098.
5. Durik, M. *et al*. (2012) Clin. Sci. (Lond.) 123:205.
6. Imai, T. *et al*. (1983) Proc. Natl. Acad. Sci. USA 80:7405.
7. Pratt, R.E. *et al*. (1987) Proc. Natl. Acad. Sci. USA 84:7837.
8. Do, Y.S. *et al*. (1987) J. Biol. Chem. 262:1037.
9. Wang, P.H. *et al*. (1991) J. Biol. Chem. 266:12633.
10. Mercure, C. *et al.* (2010) Am. J. Physiol. Regul. Integr. Comp. Physiol. 298:R1212.
11. DiBona, G.F. and M. Esler (2010) Am. J. Physiol. Regul. Integr. Comp. Physiol. 298:R245.
12. Kurtz, A. (2012) Am. J. Hypertens. Epub. PMID 22237158.
13. Glorioso, N. *et al.* (1986) Science 233:1422.
14. Goncalves, P.B. *et al.* (2012) Reproduction 143:11.
15. Nguyen, G. *et al.* (2002) J. Clin. Invest. 109:1417.
16. Batenburg, W.W. *et al.* (2007) J. Hypertens. 25:2441.
17. Nabi, A.H. *et al.* (2006) Int. J. Mol. Med. 18:483.
18. Reudelhuber, T.L. (2012) Curr. Opin. Nephrol. Hypertens. 21:137.
19. Nguyen, G. *et al.* (2011) Clin. Sci. 120:169.
20. Cousin, C. *et al.* (2009) Hypertension 53:1077.
21. Nguyen, G. *et al.* (1996) Kidney Int. 50:1897.
22. Peters, J. *et al.* (2002) Circ. Res. 90:1135.

PLATE LAYOUT

Use this plate layout to record standards and samples assayed.



产品信息及操作手册

人 Renin Valukine™ ELISA 试剂盒

目录号：VAL244

适用于定量检测天然和重组人成熟 Renin 和 pro-Renin 的浓度

科研专用，不可用于临床诊断

Bio-Techne China Co. Ltd

P: +86 (21) 52380373 P: 8009881270 F: +86 (21) 52381001

info.cn@bio-techne.com

有效期详见试剂盒包装标签

Novus 试剂盒确保在你收货日期 3 个月内有效

版本号 202411.1

目录

I. 背景

Renin 是天冬氨酰蛋白酶家族的 N-糖基化成员，在激活肾素-血管紧张素-醛固酮系统（Renin-Angiotensin-Aldosterone System, RAAS）中发挥着关键作用。RAAS 活性参与调节血压、钠平衡、炎症、免疫、细胞内氧化还原平衡、葡萄糖稳态以及脂肪细胞和造血祖细胞的功能（1-5）。Renin 与该家族其他成员的不同之处在于，它的最佳活性接近中性 pH 值，而不是 pH 值 2.0 至 3.4 的范围。这种更为中性的最佳 pH 值使 Renin 能够在血浆中发挥作用。Renin 对裂解 Serpin A8/Angiotensinogen 原具有高度特异性，Serpin A8/Angiotensinogen 是 Renin 唯一已知的生理底物。Renin 裂解血管紧张素原释放出十肽血管紧张素 I，再由血管紧张素转换酶（ACE）和 ACE-2 进一步裂解为生物活性肽血管紧张素 II、Ang1-9 和 Ang1-7。血管紧张素 II 是 RAAS 活性的强效效应因子。人 Prorenin，47 kDa，由一个 43 氨基酸（aa）的前肽和一个 340 aa 的活性酶组成，以非活性酶的形式分泌（6-8）。在血液循环中，前肽被蛋白水解去除，从而 Prorenin 被激活。另一种方法是，在细胞内去除前肽，然后分泌具有活性的 44 kDa Renin（7, 9, 10）。成熟的 Renin 可进一步裂解为 22 kDa 和 18 kDa 的片段，这些片段只要保持关联就会表现出酶活性（8）。人 Prorenin 与小鼠和大鼠 Prorenin 分别有 70%和 66%的 aa 序列相同性。有活性的人 Renin 与小鼠和大鼠 Renin 的 aa 序列相同度分别为 71%和 67%。Renin 是由肾脏中的并肾小球细胞在肾交感神经活动、低钠水平、低血压、前列腺素和多种激素的作用下产生的（1, 11, 12）。Prorenin 还在肾脏以外的多个部位表达，包括卵巢和胰腺。血管紧张素 II 的生成对卵巢的卵泡发育以及调节胰腺的葡萄糖代谢和胰岛素抵抗非常重要（3, 13, 14）。

循环中的 Prorenin 和 Renin 与 Renin 受体（Renin R）结合，Renin R 又称 V-ATPase 辅助蛋白 2（15-19）。Renin R 在肾间质细胞、冠状动脉和肾动脉内皮下层、血管平滑肌细胞、胎盘血管和合胞滋养细胞上都有表达（15）。Renin R 与 Prorenin 结合后可诱导肾素酶的活性，而不需要蛋白水解去除前肽（15, 16）。Renin R 是一种跨膜蛋白，可以被分泌为 28 kDa 的可溶性形式，并保持与 Renin 结合的能力（20）。循环中的 Prorenin 可引发间质细胞增殖，并可被心肌细胞内化（21, 22）。

II. 概述

A. 检测原理

本实验采用双抗体夹心ELISA法。抗人Renin抗体包被于微孔板上，样品和标准品中的人Renin会与固定在板上的抗体结合，游离的成分被洗去；加入辣根过氧化酶标记的抗人Renin检测抗体进行孵育。洗涤去除未结合的试剂后，加入TMB底物溶液（显色剂）。溶液颜色与结合目标蛋白成正比；加入终止液；用酶标仪测定吸光度。

B. 检测局限

- **仅供科研使用，不可用于体外诊断**。
- 该试剂盒适用于细胞培养上清液，人血清、人血浆和人尿液。
- 请在试剂盒有效期内使用。
- 不同试剂盒及不同批号试剂盒的组分不能混用。
- 样本值若大于标准曲线的最高值，应将样本用标准品稀释液（1×）稀释并重复测定。
- 检测结果的不同可由多种因素引起，包括实验人员的操作、移液器的使用方式、洗板技术、反应时间或温度、试剂盒的效期等。

III. 优势

A. 精确度

板内精确度（同一板内不同孔间的精确度）

已知浓度的三个样本，在同一板内分别检测20次，以确定板内精确度。

板间精确度（不同板之间的精确度）

已知浓度的三个样本，在不同板间分别检测20次，以确定板间精确度。

	板内精确度			板间精确度		
样本	1	2	3	1	2	3
平均值（pg/mL）	201	599	1193	191	566	1153
标准差	10.6	14.2	19.9	10.5	27.1	46.4
CV%	5.3	2.4	1.7	5.5	4.8	4.0

B. 回收率

不同类型样本中掺入检测范围内不同水平的人Renin，测定其回收率。

样本类型	平均回收率（%）	范围（%）
细胞培养基（n=8）	100	93-106
人血清*（n=4）	101	90-109
人 EDTA 血浆*（n=4）	101	95-107
人肝素血浆*（n=4）	105	99-114
人尿液*（n=4）	93	80-106

*样品在分析前进行稀释。

C. 灵敏度

评估 20 次测定，人 Renin 的最小可检测剂量（MDD）范围为 0.769-14.8 pg/mL。平均 MDD 为 4.43 pg/mL。

MDD是根据20个重复的零标准品孔的吸光度值的平均值加两倍标准差计算得到的相对应浓度。

D. 校正

该免疫测定法以R&D Systems生产的高纯度的NS0表达的重组人Pro-Renin校正。本试剂盒使用NIBSC/WHO国际Renin标准品（68/356）为效价标准。NIBSC/WHO标准品为人肾脏的纯化提取物。

国际标准品（68/356）的剂量反应曲线与Valukine标准曲线平行。要将使用Valukine人Renin试剂盒获得的样本值转换为近似的NIBSC 68/356数据，请使用以下公式。

NIBSC（68/356）近似值（IU/mL）=1.58 × 10^{-7} × Valukine人Renin值（pg/mL）

E. 线性

不同的样本中含有或掺入高浓度的人Renin，然后用标准品稀释液（1×）将样本稀释到检测范围内，测定其线性。

稀释倍数		细胞培养基（n=8）	人血清*（n=4）	人EDTA血浆*（n=4）	人肝素血浆*（n=4）	人尿液（n=4）
1:2	平均值/期待值（%）	98	101	99	101	100
	范围（%）	95-102	99-103	96-102	100-102	96-103
1:4	平均值/期待值（%）	99	106	100	102	101
	范围（%）	96-102	104-107	96-104	99-107	96-106
1:8	平均值/期待值（%）	102	108	103	102	102
	范围（%）	94-108	103-114	95-108	94-111	96-107
1:16	平均值/期待值（%）	98	105	108	106	107
	范围（%）	88-108	100-109	96-119	97-112	98-115

*样品在分析前进行稀释。

F. 样本预值

人血清/血浆/尿液 - 在本试验中评估了来自表面健康志愿者的样本中是否存在人Renin。本研究中使用的供体没有病史。

样本类型	平均值（pg/mL）	范围（pg/mL）	标准差（pg/mL）
人血清（n=35）	776	232-1892	374
人EDTA血浆（n=35）	726	201-1852	365
人肝素血浆（n=35）	680	179-1760	349

样本类型	平均检测值（pg/mL）	%可检测率	范围（pg/mL）
人尿液（n=12）	60.4	42	ND-96.7

ND=未检出

细胞培养上清 - CCD-1070Sk人真皮成纤维细胞在MEM培养基中，其中加入10%胎牛血清和1 mM丙酮酸钠，培养8天，直至汇合。取细胞培养上清液，检测人Renin，测量值为197 pg/mL。

G. 特异性

检测方法识别天然和重组人Renin。

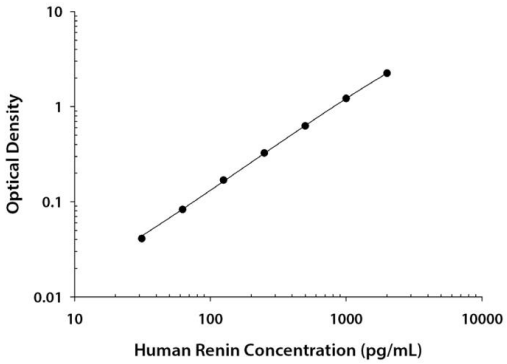
以下列出的因子在标准品稀释液（1×）中以50 ng/mL的浓度制备，并进行交叉反应性测定。以下列出的因子在中值范围重组人Renin对照品中以50 pg/mL的浓度制备，并进行干扰测定。未观察到明显的交叉反应或干扰。

Recombinant human:	**Recombinant mouse:**
ACE/CD143	ACE/CD143
ACE-2	Renin
Cathepsin B	Serpin A8/Angiotensinogen
Cathepsin D	**Recombinant rat:**
Cathepsin E	Renin
Renin R	
Serpin A8/Angiotensinogen	

IV. 实验

标准曲线实例

该标准曲线数据仅供参考，每次实验应绘制其对应的标准曲线。



(pg/mL)	O.D.	Average	Corrected
0	0.011 0.013	0.012	—
31.3	0.051 0.055	0.053	0.041
62.5	0.095 0.095	0.095	0.083
125	0.179 0.183	0.181	0.169
250	0.333 0.341	0.337	0.325
500	0.638 0.638	0.638	0.626
1000	1.220 1.241	1.231	1.219
2000	2.248 2.264	2.256	2.244

V. 试剂盒组成及储存

A. 试剂盒组成

组成	描述	规格
Human Renin Microplate	包被抗人Renin抗体的96孔聚苯乙烯板，8孔× 12条	1块板
Human Renin Conjugate	酶标检测抗人Renin抗体	1瓶
Human Renin Standard	重组人pro-Renin标准品（冻干），参考瓶身标签进行重溶	1瓶
Assay Diluent RD1S	检测液	1瓶
Calibrator Diluent Concentrate (5×)/ RD5P	浓缩的标准品稀释液（5×），用于稀释标准品和样品	1瓶
Wash Buffer Concentrate (25×)	浓缩洗涤缓冲液（25×）	1瓶
TMB Substrate	TMB ELISA底物溶液/TMB底物溶液	2瓶
Stop Solution	终止液	1瓶
Plate Sealers	封板膜	3张

B. 试剂盒储存

<table>
<tr><td>未开封试剂盒</td><td colspan="2">2-8°C储存；请在试剂盒有效期内使用</td></tr>
<tr><td rowspan="7">已打开，稀释或重溶的试剂</td><td>洗涤液（1×）</td><td rowspan="4">2-8°C储存，最多30天*</td></tr>
<tr><td>终止液</td></tr>
<tr><td>酶标检测抗体</td></tr>
<tr><td>TMB底物溶液</td></tr>
<tr><td>标准品</td><td>2-8°C储存，最多30天*</td></tr>
<tr><td>检测液RD1S</td><td>2-8°C储存，最多30天*</td></tr>
<tr><td>浓缩的标准品稀释液（5×）/ RD5P</td><td>2-8°C 储存，最多 30 天*
请每次使用新鲜配制的1×标准品稀释液，多余的丢弃</td></tr>
<tr><td>包被的微孔板条</td><td>将未用的板条放回带有干燥剂的铝箔袋内，密封；
2-8 °C储存，最多30天*</td></tr>
</table>

*必须在试剂盒有效期内

C. 实验所需自备试验器材

- 酶标仪（可测量450 nm检测波长的吸收值及540 nm或570 nm校正波长的吸收值）
- 高精度加液器及一次性吸头
- 蒸馏水或去离子水
- 洗瓶（喷瓶）、多通道洗板器或自动洗板机
- 100 mL和500 mL量筒
- 水平振荡器（0.12”轨道），转速：500±50 rpm
- 用于稀释标准品和样品的管子

D. 注意事项

- 试剂盒中的一些组分含有防腐剂，可能引起皮肤过敏反应，避免吸入。
- 试剂盒中的终止液是酸性溶液，使用时请做好眼睛、手、面部及衣服的防护。

VI. 实验前准备

A. 样品收集及储存

以下列出的样品收集和储存条件仅作为一般指南。样品稳定性尚未评估。

细胞培养上清液 - 通过离心去除颗粒物，立即或等分进行检测，并将样品储存在≤ -20°C的温度下，避免反复冻融。样品可能需要用标准品稀释液（1×）稀释。

血清 - 使用血清分离管（SST），让样本在室温下凝固30分钟，然后在1000 × g 的离心力下离心15分钟。分离血清并立即进行检测，或分装并储存在≤ -20 °C。避免反复冻融。样本可能需要用标准品稀释液（1×）稀释。

血浆 - 使用肝素EDTA作为抗凝剂收集血浆。在采样后30分钟内，以1000 × g的离心力离心15分钟。立即检测或分装并储存在≤ -20 °C。避免反复冻融。样品可能需要用标准品稀释液（1×）稀释。

注：*柠檬酸盐血浆在本检测中未经验证。*

溶血样本不建议用于此检测。

尿液 - 无菌采集当日首次尿液（中段尿），直接排入无菌容器中。离心去除颗粒物质，立即检测或分装后储存于≤ -20 °C。避免反复冻融。样品可能需要用标准品稀释液（1×）进行稀释。

B. 样品准备

人血清和血浆样品建议稀释 2 倍。建议的 2 倍稀释方式：75 µL 的样本+75 µL 的标准品稀释液（1×）。

C. 检测前准备工作

使用前请将所有试剂放置于室温。

洗涤液（1×）：从冰箱中取出的浓缩洗涤液可能有结晶，属于正常现象；放置室温，轻摇混匀，待结晶完全溶解后再配制洗涤液。可将20 mL浓缩洗涤液（25×）用蒸馏水或去离子水稀释配制成500 mL工作浓度的洗涤液（1×）。

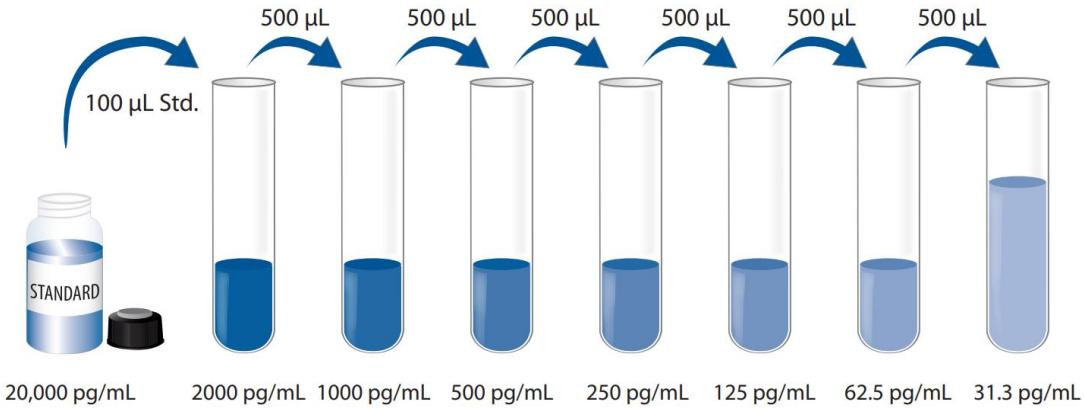
标准品稀释液（1×）：使用去离子水或蒸馏水制备标准品稀释液（1×）。

人Renin标准品：重溶体积请参考瓶身标签*，用去离子水或蒸馏水重溶人Renin标准品，得到浓度为20000 pg/mL标准品储备母液。轻轻震摇至少15分钟，其充分溶解。

*如有疑问，请咨询我们的技术支持。

用移液管将900 µL标准品稀释液（1×）移入2000 pg/mL的管中，剩余管中加入500 µL标准品稀释液（1×）。使用储备品母液稀释（如下）。在下次转移之前，将每个管彻底混合。2000 pg/mL标准品作为标准曲线的最高点，标准品稀释液（1×）作为标准曲线

的零点（0 pg/mL）。



D. 技术小提示

- 当混合或重溶蛋白液时，尽量避免起沫。
- 为了避免交叉污染，配制不同浓度标准品、上样、加不同试剂都需要更换枪头。另外不同试剂请分别使用不同的移液槽。
- 建议15分钟内完成一块板的上样。
- 每次孵育时，正确使用封板膜可保证结果的准确性。
- TMB底物溶液在上板前应为无色，请避光保存；加入微孔板后，将由无色变成不同深度的蓝色。
- 终止液上板顺序应同TMB底物溶液上板顺序一致；加入终止液后，孔内颜色由蓝变黄；若孔内有绿色，则表明孔内液体未混匀请充分混合。

VII.操作步骤

使用前，将所有其他试剂和样品放置于室温。建议对所有标准品和样品进行复孔检测。

1. 按照上一节的说明，准备好所有需要的试剂和标准品。
2. 从已平衡至室温的密封袋中取出微孔板，未用的板条请放回铝箔袋内，重新封口。
3. 向每个孔中加入100 µL的检测液RD1S。
4. 分别将不同浓度标准品和实验样本加入相应孔中，每孔50 µL。用封板膜封住反应孔，**用水平振荡器（0.12”轨道），转速：500±50 rpm，室温孵育2小时**。说明书提供了一张96孔模板图，可用于记录标准品和试验样本的板内位置。
5. 将板内液体吸去，使用洗瓶、多通道洗板器或自动洗板机洗板。每孔加洗涤液400 µL，然后将板内洗涤液吸去。重复操作3次，共洗4次。每次洗板尽量吸去残留液体会有助于得到好的实验结果。最后一次洗板结束，请将板内所有液体吸干或将板倒置，在吸水纸拍干所有残留液体。
6. 在每个微孔内加入200 µL人Renin酶标检测抗体。用新的封板膜封住反应孔，**用水平振荡器（0.12”轨道），转速：500±50 rpm，室温孵育2小时**。
7. 重复第5步洗板操作。
8. 在每个微孔内加入200 µL TMB底物溶液，**室温孵育30分钟。注意避光**。
9. 在每个微孔内加入50 µL终止液，请轻拍微孔板，使溶液混合均匀。
10. 加入终止液后10分钟内，使用酶标仪测量450 nm的吸光度值，设定540 nm或570 nm作为校正波长。如果波长校正不可用，以450 nm的读数减去540 nm或570 nm的读数。这种减法将校正酶标板上的光学缺陷。没有校正而直接在450 nm处进行的读数可能会更高且更不准确。
11. 计算结果：

    将每个标准品和样品的复孔吸光值取平均值，然后减去零标准品平均OD值（O.D.），使用计算机软件作四参数逻辑（4-PL）曲线拟合创建标准曲线。

    另一替代方法是，通过绘制y轴上每个标准品的平均吸光值与x轴上的浓度来构建标准曲线，并通过图上的点绘制最佳拟合曲线。数据可以通过绘制人Renin浓度的对数与O.D.的对数来线性化，并且最佳拟合线可以通过回归分析来确定。该程序将产生足够但不太精确的数据拟合。

    如果样品被稀释，从标准曲线读取的浓度必须乘以稀释倍数。

VIII. 参考文献

1. Castrop, H. *et al*. (2010) Physiol. Rev. 90:607.
2. Capettini, L.S.A. *et al*. (2012) Curr. Pharmaceut. Des. 18:963.
3. Luther, J.M. and N.J. Brown (2011) Trends Pharmacol. Sci. 32:734.
4. Jing, F. *et al.* (2012) Mol. Cell. Endocrinol. Epub. PMID 22465098.
5. Durik, M. *et al*. (2012) Clin. Sci. (Lond.) 123:205.
6. Imai, T. *et al*. (1983) Proc. Natl. Acad. Sci. USA 80:7405.
7. Pratt, R.E. *et al*. (1987) Proc. Natl. Acad. Sci. USA 84:7837.
8. Do, Y.S. *et al*. (1987) J. Biol. Chem. 262:1037.
9. Wang, P.H. *et al*. (1991) J. Biol. Chem. 266:12633.
10. Mercure, C. *et al.* (2010) Am. J. Physiol. Regul. Integr. Comp. Physiol. 298:R1212.
11. DiBona, G.F. and M. Esler (2010) Am. J. Physiol. Regul. Integr. Comp. Physiol. 298:R245.
12. Kurtz, A. (2012) Am. J. Hypertens. Epub. PMID 22237158.
13. Glorioso, N. *et al.* (1986) Science 233:1422.
14. Goncalves, P.B. *et al.* (2012) Reproduction 143:11.
15. Nguyen, G. *et al.* (2002) J. Clin. Invest. 109:1417.
16. Batenburg, W.W. *et al.* (2007) J. Hypertens. 25:2441.
17. Nabi, A.H. *et al.* (2006) Int. J. Mol. Med. 18:483.
18. Reudelhuber, T.L. (2012) Curr. Opin. Nephrol. Hypertens. 21:137.
19. Nguyen, G. *et al.* (2011) Clin. Sci. 120:169.
20. Cousin, C. *et al.* (2009) Hypertension 53:1077.
21. Nguyen, G. *et al.* (1996) Kidney Int. 50:1897.
22. Peters, J. *et al.* (2002) Circ. Res. 90:1135.

96 孔模板图

请使用 96 孔模板图来记录标准品及样本在板内的位置