Quantikine® ELISA

Human Renin Immunoassay

Catalog Number DREN00

For the quantitative determination of mature and pro- Renin concentrations in cell culture supernates, serum, plasma, and urine.

This package insert must be read in its entirety before using this product.
For research use only. Not for use in diagnostic procedures.

TABLE OF CONTENTS

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INTRODUCTION

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).

The Quantikine Human Renin Immunoassay is a 4.5 hour solid-phase ELISA designed to measure human mature and pro-renin in cell culture supernates, serum, plasma, and urine. It contains NS0-expressed recombinant human Renin and has been shown to accurately quantitate the recombinant factor. Results obtained using natural human Renin showed linear curves that were parallel to the standard curves obtained using the Quantikine kit standards. These results indicate that this kit can be used to determine relative mass values for naturally occurring human Renin.

PRINCIPLE OF THE ASSAY

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

LIMITATIONS OF THE PROCEDURE

- FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.
- 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, further dilute the samples with Calibrator Diluent and repeat the assay.
- Any variation in standard diluent, operator, pipetting technique, washing technique, incubation time or temperature, and kit age can cause variation in binding.
- Variations in sample collection, processing, and storage may cause sample value differences.
- This assay is designed to eliminate interference by other factors present in biological samples. Until all factors have been tested in the Quantikine Immunoassay, the possibility of interference cannot be excluded.

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.
- To ensure accurate results, proper adhesion of plate sealers during incubation steps is necessary.
- When using an automated plate washer, adding a 30 second soak period following the addition of Wash Buffer, and/or rotating the plate 180 degrees between wash steps may improve assay precision.
- Substrate Solution should remain colorless until added to the plate. Keep Substrate Solution protected from light. Substrate Solution should change from colorless to gradations of blue.
- Stop Solution should be added to the plate in the same order as the Substrate Solution. 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 Substrate Solution.

MATERIALS PROVIDED & STORAGE CONDITIONS

Store the unopened kit at 2-8 °C. Do not use past kit expiration date.

PART	PART #	DESCRIPTION	STORAGE OF OPENED/ RECONSTITUTED MATERIAL
Human Renin Microplate	894353	96 well polystyrene microplate (12 strips of 8 wells) coated with a monoclonal antibody specific for human Renin.	Return unused wells to the foil pouch containing the desiccant pack. Reseal along entire edge of the zip-seal. May be stored for up to 1 month at 2-8 °C.*
Human Renin Conjugate	894354	21 mL of monoclonal antibody specific for human Renin conjugated to horseradish peroxidase with preservatives.	May be stored for up to 1 month at 2-8 °C.*
Human Renin Standard	894355	20 ng of recombinant human pro-Renin in a buffered protein base with preservatives; lyophilized.	
Assay Diluent RD1S	895137	11 mL of a buffered protein base with preservatives.	
Calibrator Diluent RD5P Concentrate	895151	21 mL of a concentrated buffered protein base with preservatives. *Use diluted 1:5 in this assay.*	
Wash Buffer Concentrate	895003	21 mL of a 25-fold concentrated solution of buffered surfactant with preservative. *May turn yellow over time.*	
Color Reagent A	895000	12 mL of stabilized hydrogen peroxide.	
Color Reagent B	895001	12 mL of stabilized chromogen (tetramethylbenzidine).	
Stop Solution	895032	6 mL of 2 N sulfuric acid.	
Plate Sealers	N/A	4 adhesive strips.	

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

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 cylinders.
- Horizontal orbital microplate shaker (0.12" orbit) capable of maintaining a speed of 500 ± 50 rpm.
- Test tubes for dilution of standards and samples.
- Human Renin Controls (optional; R&D Systems, Catalog # QC154).

PRECAUTIONS

The Stop Solution provided with this kit is an acid solution.

Some components in this kit contain ProClin® which may cause an allergic skin reaction. Avoid breathing mist.

Color Reagent B may cause skin, eye, and respiratory irritation. Avoid breathing fumes.

Wear protective gloves, clothing, eye, and face protection. Wash hands thoroughly after handling. Please refer to the MSDS on our website prior to use.

SAMPLE COLLECTION & 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.

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 x g. Remove serum and assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

Plasma - Collect plasma using heparin or EDTA as an anticoagulant. Centrifuge for 15 minutes at 1000 x g within 30 minutes of collection. Assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

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.

SAMPLE PREPARATION

Serum and plasma samples require a 2-fold dilution. A suggested 2-fold dilution is 75 µL of sample + 75 µL of Calibrator Diluent RD5P (diluted 1:5)*.

*See Reagent Preparation section.

REAGENT PREPARATION

Bring all reagents to room temperature before use.

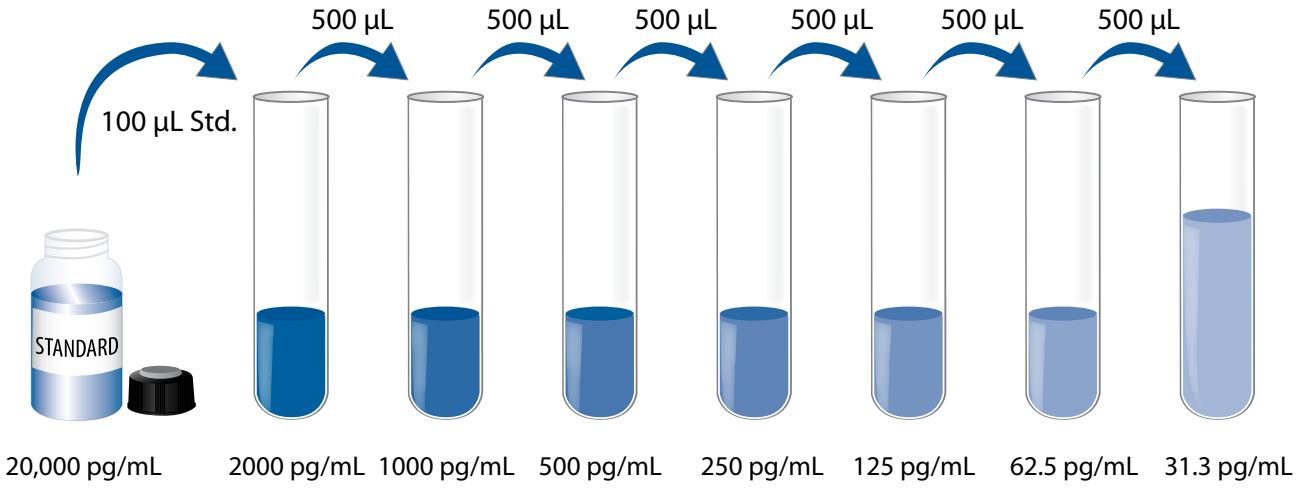
Wash Buffer - If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. Add 20 mL of Wash Buffer Concentrate to deionized or distilled water to prepare 500 mL of Wash Buffer.

Substrate Solution - Color Reagents A and B should be mixed together in equal volumes within 15 minutes of use. Protect from light. 200 µL of the resultant mixture is required per well.

Calibrator Diluent RD5P (diluted 1:5) - Add 10 mL of Calibrator Diluent RD5P Concentrate to 40 mL of deionized or distilled water to yield 50 mL of Calibrator Diluent RD5P (diluted 1:5).

Human Renin Standard - Reconstitute the Human Renin Standard with 1.0 mL of deionized or distilled water. This reconstitution produces a stock solution of 20,000 pg/mL. Mix the standard to ensure complete reconstitution and allow the standard to sit for a minimum of 15 minutes. Mix well prior to making dilutions.

Pipette 900 µL of Calibrator Diluent RD5P (diluted 1:5) 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 RD5P (diluted 1:5) serves as the zero standard (0 pg/mL).



ASSAY PROCEDURE

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

1. Prepare all reagents, working standards, and samples 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, control, or sample* per well. Cover with the adhesive strip provided. Incubate for 2 hours at room temperature on a horizontal orbital microplate shaker (0.12" orbit) set at 500 ± 50 rpm.
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 the shaker.
7. Repeat the aspiration/wash as in step 5.
8. Add 200 µL of Substrate Solution to each well. Incubate for 30 minutes at room temperature **on the benchtop. Protect from light.**
9. Add 50 µL of Stop Solution to each well. The color in the wells should change from blue to yellow. If the color in the wells is green or the color change does not appear uniform, gently tap the plate to ensure thorough mixing.
10. Determine the optical density of each well within 30 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.

*Samples may require dilution. See Sample Preparation section.

CALCULATION OF RESULTS

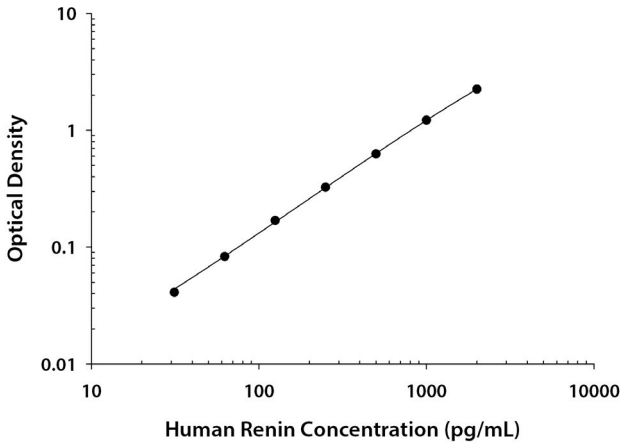
Average the duplicate readings for each standard, control, and sample and subtract the average zero standard optical density (O.D.).

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.

TYPICAL DATA

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

PRECISION

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

Inter-assay Precision (Precision between assays)
Three samples of known concentration were tested in twenty separate assays to assess inter-assay precision. Assays were performed by at least three technicians using two lots of components.

	Intra-Assay Precision			Inter-Assay Precision		
Sample	1	2	3	1	2	3
n	20	20	20	20	20	20
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

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%
Serum* (n=4)	101	90-109%
EDTA plasma* (n=4)	101	95-107%
Heparin plasma* (n=4)	105	99-114%
Urine (n=4)	93	80-106%

*Samples were diluted prior to assay.

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.

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 Quantikine standard curve. To convert sample values obtained with the Quantikine Human Renin kit to approximate NIBSC 68/356 units, use the equation below.

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

LINEARITY

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

		Cell culture media (n=8)	Serum* (n=4)	EDTA plasma* (n=4)	Heparin plasma* (n=4)	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.

SAMPLE VALUES

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)
Serum (n=35)	776	232-1892	374
EDTA plasma (n=35)	726	201-1852	365
Heparin plasma (n=35)	680	179-1760	349

Sample Type	Mean of Detectable (pg/mL)	% Detectable	Range (pg/mL)
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.

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 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:

ACE/CD143
ACE-2
Cathepsin B
Cathepsin D
Cathepsin E
Renin R
Serpin A8/Angiotensinogen

Recombinant mouse:

ACE/CD143
Renin
Serpin A8/Angiotensinogen

Recombinant rat:

Renin

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