Renin Inhibitor Screening Assay Kit

Item No. 10006270

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TABLE OF CONTENTS

GENERAL INFORMATION
3 Materials Supplied
4 Safety Data
4 Precautions
4 If You Have Problems
4 Storage and Stability
4 Materials Needed but Not Supplied

INTRODUCTION
5 Background
5 About This Assay

PRE-ASSAY PREPARATION
6 Reagent Preparation

ASSAY PROTOCOL
7 Plate Set Up
9 Performing the Assay

ANALYSIS
10 Calculations
11 Performance Characteristics

RESOURCES
12 Interferences
13 Troubleshooting
13 References
14 Plate Template
15 Notes
15 Warranty and Limitation of Remedy

GENERAL INFORMATION

Materials Supplied

Item Number	Item	Quantity
10006870	Renin Assay Buffer (10X)	1 vial
10006871	Renin (human recombinant) Assay Reagent	1 vial
10006872	Renin Substrate	1 vial
400017	96-Well Plate (black)	1 plate
400012	96-Well Cover Sheet	1 cover

If any of the items listed above are damaged or missing, please contact our Customer Service department at (800) 364-9897 or (734) 971-3335. We cannot accept any returns without prior authorization.

WARNING: THIS PRODUCT IS FOR RESEARCH ONLY - NOT FOR HUMAN OR VETERINARY DIAGNOSTIC OR THERAPEUTIC USE.

Safety Data

This material should be considered hazardous until further information becomes available. Do not ingest, inhale, get in eyes, on skin, or on clothing. Wash thoroughly after handling. Before use, the user must review the complete Safety Data Sheet, which has been sent *via* email to your institution.

Precautions

Please read these instructions carefully before beginning this assay.

If You Have Problems

Technical Service Contact Information

Phone: 888-526-5351 (USA and Canada only) or 734-975-3888

Fax: 734-971-3641

Email: techserv@caymanchem.com

Hours: M-F 8:00 AM to 5:30 PM EST

In order for our staff to assist you quickly and efficiently, please be ready to supply the lot number of the kit (found on the outside of the box).

Storage and Stability

This kit will perform as specified if stored as directed at -80°C and used before the expiration date indicated on the outside of the box.

Materials Needed But Not Supplied

1. A fluorometer with the capacity to measure fluorescence using excitation wavelengths of 335-345 nm and emission wavelengths of 485-510 nm
2. Adjustable pipettes and a repeating pipettor
3. A source of UltraPure water (Milli-Q or HPLC-grade water)

INTRODUCTION

Background

Renin is an aspartyl protease of approximately 40 kDa.[1] It is released in an active form from the renal juxtaglomerular cells in response to physiologic factors, including sodium depletion, decreased blood volume and blood pressure, and β-adrenergic stimulation.[2,3] Renin converts angiotensinogen into angiotensin I. Angiotensin converting enzyme (ACE), a monomeric zinc metalloenzyme found in the vascular endothelium, then converts this pro-hormone to angiotensin II, the final active messenger in the renin-angiotensin system (RAS) pathway.[2,3] Angiotensin II inhibits renin secretion by acting directly on the juxtaglomerular cells. Angiotensin II has a number of physiological effects, most importantly as a powerful vasoconstrictor, increasing blood pressure by altering peripheral vascular resistance. Since angiotensinogen is the only known substrate for renin and cleavage of angiotensinogen by renin is the rate determining step in the RAS pathway, it is of general consensus that inhibition of renin would be an attractive strategy for the control of hypertension. Furthermore, renin inhibitors would prevent the formation of angiotensin I and angiotensin II, and, therefore, may act differently from angiotensin receptor blockers and ACE inhibitors, which increase angiotensin I levels but do not block ACE-independent angiotensin II production.

About This Assay

Cayman's Renin Inhibitor Screening Assay Kit provides a convenient method for screening human renin inhibitors. The assay utilizes a synthetic peptide substrate. The peptide, which is the normal substrate for renin, has been linked to a fluorophore (EDANS) at one end and to a nonfluorescent chromophore (Dabcyl) at the other.[4] After cleavage by renin, the product (peptide-EDANS) is brightly fluorescent and can be easily analyzed using a fluorescence plate reader or a fluorometer with excitation wavelengths of 335-345 nm and emission wavelengths of 485-510 nm.

PRE-ASSAY PREPARATION

Reagent Preparation

1. **Renin Assay Buffer (10X) - (Item No. 10006870)**

   Dilute 3 ml of Assay Buffer concentrate with 27 ml of HPLC-grade water. This final Assay Buffer (50 mM Tris-HCl, pH 8.0, containing 100 mM NaCl) should be used in the assay and for dilution of Renin. When stored at -80°C, this diluted Assay Buffer is stable for at least six months.

2. **Renin (human recombinant)**

   Assay Reagent - (Item No. 10006871)

   The vial contains a solution of human recombinant renin. Store the thawed enzyme on ice. Prior to assaying, dilute 50 µl of enzyme with 950 µl of diluted Assay Buffer. This is sufficient enzyme for the full 96-well plate. If not utilizing the entire plate, adjust the amount of diluted enzyme accordingly by diluting the enzyme 1:20 with Assay Buffer before use. The diluted enzyme is stable for four hours on ice. Prepare aliquots of the remainder of the undiluted enzyme and store at -80°C.

3. **Renin Substrate - (Item No. 10006872)**

   The vial contains a 95 µM solution of Arg-Glu(EDANS)-Ile-His-Pro-Phe-His-Leu-Val-Ile-His-Thr-Lys(Dabcyl)-Arg in dimethylsulfoxide (DMSO). The Substrate is ready to use as supplied. *NOTE: The K_m value for the Substrate is 1.5-3.6 µM for human recombinant Renin.[4,5] The final concentration of Substrate in the assay as described below is 10 µM. This concentration may be reduced with DMSO at the user's discretion, particularly when complete inhibition curves are required for IC_{50} or K_i determination. For competitive inhibitors, the IC_{50} is dependent upon the Substrate concentration and should be reported when publishing the experimental results.*

ASSAY PROTOCOL

Plate Set Up

There is no specific pattern for using the wells on the plate. However, it is necessary to have three wells designated as background wells and three wells designated as 100% Initial Activity wells. We suggest that each Inhibitor sample be assayed in triplicate and you record the contents of each well on the template sheet provided on page 14. A typical layout of samples and inhibitors to be measured in triplicate is given below.

	1	2	3	4	5	6	7	8	9	10	11	12
A	BW	BW	BW	7	7	7	15	15	15	23	23	23
B	A	A	A	8	8	8	16	16	16	24	24	24
C	1	1	1	9	9	9	17	17	17	25	25	25
D	2	2	2	10	10	10	18	18	18	26	26	26
E	3	3	3	11	11	11	19	19	19	27	27	27
F	4	4	4	12	12	12	20	20	20	28	28	28
G	5	5	5	13	13	13	21	21	21	29	29	29
H	6	6	6	14	14	14	22	22	22	30	30	30

BW - Background Wells
A - 100% Initial Activity Wells
1-30 - Inhibitor Wells

Figure 1. Inhibitor screening plate format

Pipetting Hints

- It is recommended that an adjustable pipette be used to deliver reagents to the wells.
- Use different tips to pipette enzyme and Substrate.
- Before pipetting each reagent, equilibrate the pipette tip in that reagent (*i.e.*, slowly fill the tip and gently expel the contents, repeat several times).
- Do not expose the pipette tip to the reagent(s) already in the well.

General Information

- The final volume of the assay is 190 µl in all the wells.
- It is not necessary to use all the wells on the plate at one time.
- The assay temperature is 37°C. Pre-warm the Assay Buffer to 37°C before assaying. Renin activity decreases 80% if assayed at 22°C rather than at 37°C.
- If the appropriate inhibitor concentration is not known, it may be necessary to assay at several dilutions.
- We recommend assaying samples in triplicate, but it is the user's discretion to do so.
- Thirty inhibitor samples can be assayed in triplicate or forty-five in duplicate.

Performing the Assay

1. **Background Wells** - add 20 µl of Substrate, 160 µl of Assay Buffer, and 10 µl of solvent (which ever solvent you dissolved your inhibitor in) to three wells.
2. **100% Initial Activity Wells** - add 20 µl of Substrate, 150 µl of Assay Buffer, and 10 µl of solvent (which ever solvent you dissolved your inhibitor in) to three wells.
3. **Inhibitor Wells** - add 20 µl of Substrate, 150 µl of Assay Buffer, and 10 µl of inhibitor* to three wells.
4. Initiate the reactions by adding 10 µl of renin to the 100% Initial Activity and Inhibitor wells being used. Carefully shake the microwell plate for 10 seconds to mix and cover with the plate cover. Incubate for 15 minutes at 37°C.
5. Remove the plate cover and read the fluorescence using excitation wavelengths of 335-345 nm and emission wavelengths of 485-510 nm.

*Inhibitors can be dissolved in methanol, DMSO, or ethanol and should be added to the assay in a final volume of 10 µl. In the event that the appropriate concentration of inhibitor is completely unknown, we recommend that several dilutions of the inhibitor be analyzed.

ANALYSIS

Calculations

1. Determine the average fluorescence (AF) of all the samples.
2. Subtract the background AF from the 100% Initial Activity and Inhibitor AFs.
3. Use the following equation to calculate the percent inhibition:

$$\%\text{Inhibition} = \left[\frac{\text{100\% Initial Activity (AF) - Inhibitor (AF)}}{\text{100\% Initial Activity (AF)}} \right] \times 100$$

4. Either graph the Percent Inhibition or Percent Initial Activity as a function of the Inhibitor concentration to determine the IC_{50} value (concentration at which there is 50% inhibition). An example of human recombinant renin inhibition by a specific renin peptide inhibitor (Z-Arg-Arg-Pro-Phe-His-Sta-Ile-His-Lys(Boc)-OMe) is shown in Figure 2.[6]

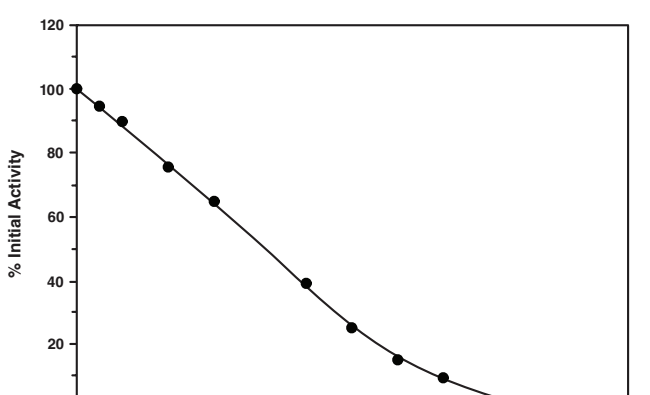

Figure 2. Inhibition of human recombinant renin by Z-Arg-Arg-Pro-Phe-His-Sta-Ile-His-Lys(Boc)-OMe (IC_{50} = 400 nM)

Performance Characteristics

Precision: Intra-assay coefficient of variation = 2.9% (n = 75). Inter-assay coefficient of variation = 7.3% (n = 5).

RESOURCES

Interferences

The following reagents were tested for interference in the assay.

	Reagent	Will Interfere (Yes or No)
Buffers:	Tris	No
	Borate	No
	Phosphate	No
Detergents/Chelators:	EGTA (≤1 mM)	No
	EDTA (≤1 mM)	No
	Polysorbate (≤ 1%)	No
	Triton X-100 (≤ 1%)	No
Solvents:	Ethanol (10 µl)	No
	Methanol (10 µl)	No
	Dimethylsulfoxide (10 µl)	No
Others:	Glycerol (≤ 5%)	No
	Bovine serum albumin (≤0.1 %)	No

Troubleshooting

Problem	Possible Causes	Recommended Solutions
Erratic values; dispersion of duplicates/triplicates	A. Poor pipetting/technique B. Bubble in the well(s)	A. Be careful not to splash the contents of the wells B. Carefully tap the side of the plate with your finger to remove bubbles
No fluorescence above background is seen in the Inhibitor wells	A. Enzyme or substrate was not added to the well(s) B. Inhibitor concentration is too high resulting in complete loss of enzyme activity	A. Make sure to add all components to the wells B. Reduce the concentration of the inhibitor and re-assay
No inhibition seen with inhibitor	The inhibitor concentration is not high enough or the compound is not an inhibitor of the enzyme	Increase the inhibitor concentration and re-assay

References

1. Imai, T., Miyazaki, H., Hirose, S., *et al. Proc. Natl. Acad. Sci. USA* **80**, 7405-7409 (1983).
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3. Persson, P.B. *J. Physiol.* **552.3**, 667-671 (2003).
4. Wang, G.T., Chung, C.C., Holzman, T.F., *et al. Anal. Biochem.* **210**, 351-359 (1993).
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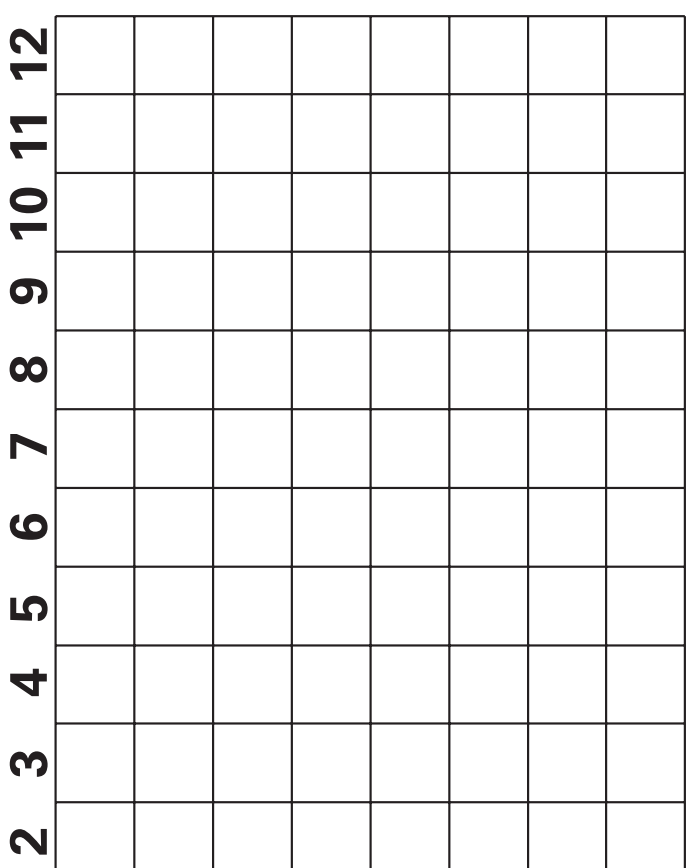
6. Wood, J.M., Gulati, N., Forgiarini, P., *et al. Hypertension* **7**, 797-803 (1985).



NOTES

Warranty and Limitation of Remedy

Buyer agrees to purchase the material subject to Cayman's Terms and Conditions. Complete Terms and Conditions including Warranty and Limitation of Liability information can be found on our website.