Renin Inhibitor Screening Assay Kit

Catalog Number KA1361

96 assays

Version: 02

Intended for research use only

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\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.

Principle of the Assay

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.

General Information

Materials Supplied

List of component

Component	Amount
Renin Assay Buffer (10X)	1 vial
Renin (human recombinant) Assay Reagent	1 vial
Renin Substrate	1 vial
96-Well Plate (black)	1 plate
96-Well Plate Cover Sheet	1 cover

Storage Instruction

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 Required but Not Supplied

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

Precautions for Use

✓ 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.

✓ 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. A typical layout of samples and inhibitors to be measured in triplicate is given in Plate Layout.

Assay Protocol

Reagent Preparation

- ✓ Renin Assay Buffer (10X) - 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.
- ✓ Renin (human recombinant) - 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.
- ✓ Renin Substrate - 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 dimethyl sulfoxide (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 Procedure

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 microtiter 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.

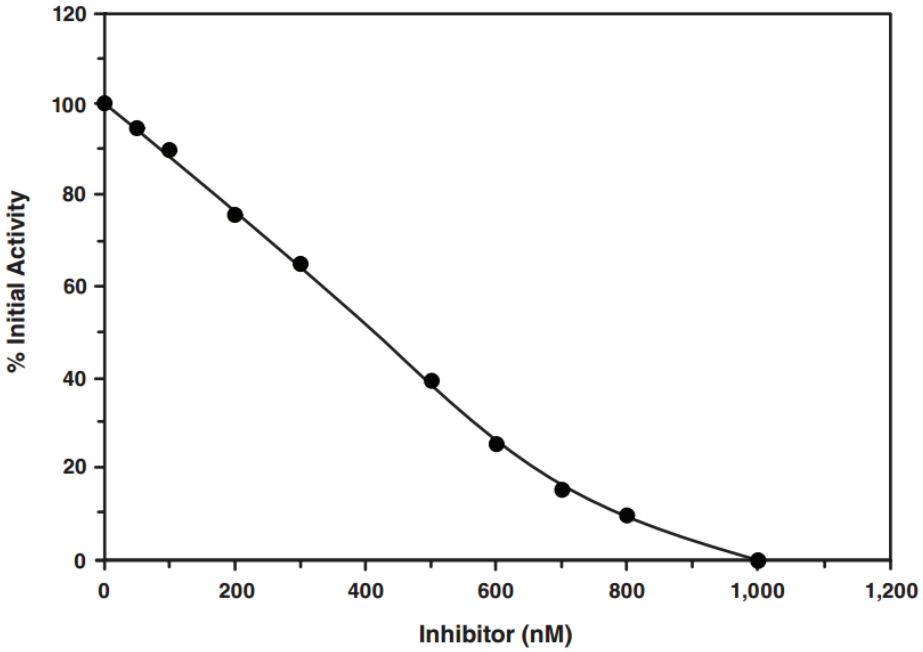
Data Analysis

Calculation of Results

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:

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

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]



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).

✓ 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
	Tween 20 (≤ 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

Resources

Troubleshooting

Problem	Possible Causes	Recommended Solutions
Erratic values; dispersion of duplicates/triplicates	A. Poor pipetting/technique B. Bubble in the well(s)	A. Carefully tap the side of the plate with your finger to remove bubbles B. Be careful not to splash the contents of the wells
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

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2. Cartledge, S. and Lawson, N. Aldosterone and renin measurements. *Ann. Clin. Biochem.* 37, 262-278 (2000).
3. Persson, P.B. Renin: Origin, secretion and synthesis. *J. Physiol.* 552.3, 667-671 (2003).
4. Wang, G.T., Chung, C.C., Holzman, T.F., *et al.* A continuous fluorescence assay of renin activity. *Anal. Biochem.* 210, 351-359 (1993).
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Plate Layout

	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