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Data Sheet

Renin Assay Kit

Catalog # 80211

DESCRIPTION: Renin is a protease that is synthesized by the kidneys. Renin catalyzes the hydrolysis of angiotensinogen to angiotensin I, which is converted to angiotensin II, a highly potent vasoconstrictor. Renin inhibitors have been used therapeutically in the treatment of hypertension (high blood pressure). The *Renin Assay Kit* is designed to measure Renin activity for screening and profiling applications. It comes in a convenient 96-well format, with purified, activated renin, renin substrate, and assay buffer for 100 enzyme reactions. Aliskiren Hydrochloride is also included as a control for renin inhibition. The key to the Renin Assay Kit is the specific, fluorogenic substrate. Using this kit, only one simple step on a microtiter plate is required for renin reactions. The fluorometric substrate is incubated with a sample containing renin enzyme to produce a fluorophore that can then be measured using a fluorescence reader.

COMPONENTS:

Catalog #	Component	Amount	Storage	
80200	Activated Renin	2 µg	-80°C	***(Avoid freeze/thaw cycles!)***
	Renin Assay Buffer	25 ml	-20°C	
	Renin Substrate	30 µl @ 1 mM	-80°C	
27303	Aliskiren Hydrochloride	20 µl @ 100µM in DMSO	-20°C	
	Black 96-well plate		RT	

MATERIALS OR INSTRUMENTS REQUIRED BUT NOT SUPPLIED:
Fluorescent microplate reader

APPLICATIONS: Great for studying enzyme kinetics, screening small molecular inhibitors for drug discovery and HTS applications.

STABILITY: 6 months from date of receipt when stored as directed.

REFERENCE(S):

1. Paschalidou, K. *et al., Biochem. J.* 2004*;* **382**: 1031.
2. Gossas, T., *et al., Naunyn Schmiedebergs Arch Pharmacol.* 2012; **385(2)**:219-24.

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ASSAY PROTOCOL:
All samples and controls should be tested in duplicate.

Step 1:

1) Dilute the Renin Substrate 1:100 using the Renin Assay Buffer. For example, add 30 µl of Renin Substrate to 2,970 µl of Renin Assay Buffer.

2) Add 25 µl of diluted Renin Substrate to each well designated as “Positive Control”, “Test Inhibitor”, "Inhibitor Control", and “Blank”.

3) Dilute Aliskiren HCl (100 µM stock) 10-fold with Renin Assay Buffer to make a 10 µM solution. Make a series of dilutions of the 10 µM solution with 10% DMSO (prepared in Renin Assay Buffer) for IC50 assay. You can use the same method to prepare the test inhibitor.

4) Add 5 µl of the prepared test inhibitor solution to each well designated “Test Inhibitor”. For the “Positive Control” and “Blank”, add 5 µl of the same solution without the inhibitor (Inhibitor buffer). For the "Inhibitor Control", add 5 µl of the Aliskiren HCl solution.

5) Thaw the human recombinant Renin on ice. Upon the first thaw, briefly spin the tube containing the enzyme to recover the full contents of the tube. Aliquot the Renin enzyme into single-use aliquots. Store the remaining undiluted enzyme in aliquots at - 80°C immediately. *Note: Renin is very sensitive to freeze/thaw cycles. Do not re-use thawed aliquots or diluted enzyme.*

	Blank	Positive Control	Inhibitor Control	Test Inhibitor
Renin Substrate (10 µM)	25 µl	25 µl	25 µl	25 µl
Test Inhibitor				5 µl
Renin Assay Buffer	20 µl			
Aliskiren HCl			5 µl	
Inhibitor Buffer (no inhibitor)	5 µl	5 µl		
Human recombinant Renin		20 µl	20 µl	20 µl
Total	50 µl	50 µl	50 µl	50 µl

6) Dilute the human recombinant Renin to 0.1 ng/µl using the Renin Assay Buffer. Add 20 µl of diluted Renin to each well designated “Positive Control”, “Test Inhibitor”, and "Inhibitor Control". For the “Blank”, add 20 µl of Renin Assay Buffer.

7) Incubate the plate at room temperature for 30 minutes.

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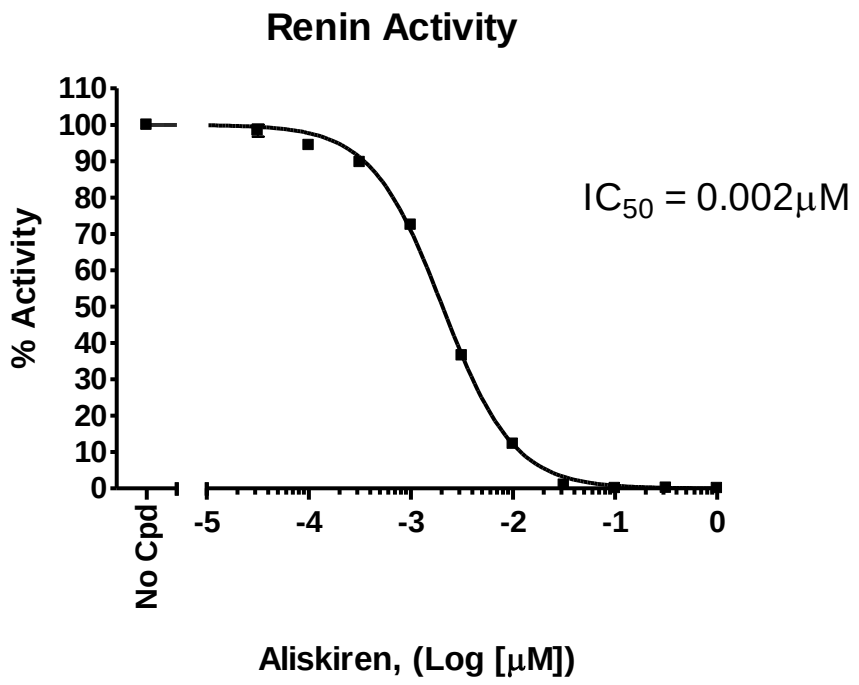
Step 2:

Read the sample in a microtiter-plate fluorimeter that is capable of excitation at wavelengths 490 nm and detection of emitted light at 520 nm. The "Blank" value is subtracted from all other values.

Example of Assay Results:



Inhibition of Activated Renin (BPS Bioscience, #80200) by Aliskiren Hydrochloride (BPS Bioscience, #27303), measured using the *Renin Assay Kit* (BPS Bioscience, #80211). Fluorescence intensity was measured using a Tecan fluorescent microplate reader.
Data shown is lot-specific. For lot-specific information, please contact BPS Bioscience, Inc. at info@bpsbioscience.com

RELATED PRODUCTS

Product Name	Catalog#	Size
Activated Renin	80200	100 µg
Aliskiren Hydrochloride	27303	1 mg