ab138875

Renin Assay Kit

(Fluorometric)

Instructions for Use

This kit provides a convenient assay for high throughput screening of renin inhibitors and for continuous assay of renin activity.

This product is for research use only and is not intended for diagnostic use.

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1. Introduction

Renin is an enzyme that participates in the renin-angiotensin system (RAS) that mediates extracellular volume, and arterial vasoconstriction. It regulates blood pressure and electrolyte homoeostasis. At the first and rate-limiting step of the RAS cascade, renin cleaves angiotensinogen to yield angiotensin I, which is further converted into angiotensin II by Angiotensin Converting Enzyme (ACE). Angiotensin II constricts blood vessels leading to increased blood pressure. It also increases the secretion of ADH and aldosterone, and stimulates the hypothalamus to activate the thirst reflex. An over-active renin-angiotension system leads to vasoconstriction and retention of sodium and water. These effects lead to hypertension. Thus, renin is an attractive target for the treatment of this disease.

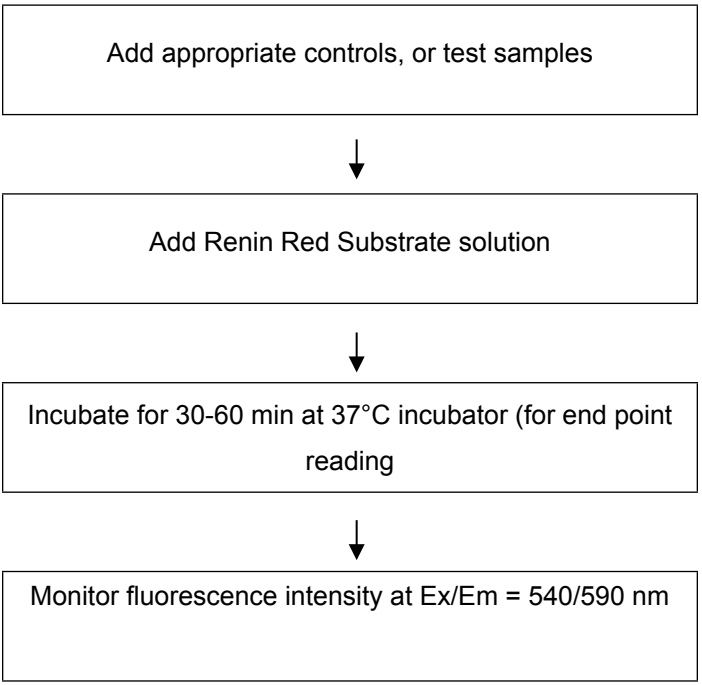
ab138875 provides a convenient assay for high throughput screening of renin inhibitors and for continuous assay of renin activity using a TF3/TQ3 FRET peptide. In the FRET peptide the fluorescence of TF3 is quenched by TQ3. Upon cleavage into two separate fragments by renin, the fluorescence of TF3 is recovered, the fluorescent signal can be easily monitored by a fluorescence microplate reader at Ex/Em = 540/590 nm. This assay is about fifty fold more sensitive than an EDANS/DABCYL-based assay. With Abcam's Renin Assay Kit (Fluorometric), we have detected as little as 1ng renin in a 100 µL reaction volume.

Kit Key Features

- **Convenient Format:** Includes all the key assay components.
- **Optimized Performance:** Optimized for detecting renin activities and screening its inhibitors.
- **Continuous:** Easily adapted to automation without a separation step.
- **Convenient:** Formulated to have minimal hands-on time. No wash is required.
- **Non-Radioactive:** No special requirements for waste treatment

2. Protocol Summary

Summary for One 96-well Plate



Note: Thaw all the kit components to room temperature before starting the experiment.

3. Kit Contents

Components	Amount
Component A: Renin Red Substrate	50 µL
Component B: Renin Standard	1 vial (40 µg/mL, 25 µL)
Component C: Assay Buffer	1 bottle (10 mL)

4. Storage and Handling

Keep at -20°C. Avoid exposure to light.

5. Additional Materials Required

- 96 or 384-well microplates: solid black microplates
- Fluorescent microplate reader

6. Assay Protocol

Note: This protocol is for one 96 - well plate.

A. Preparation

1. Prepare Renin containing biological samples as desired.

2. Prepare Renin Assay Mixture: Dilute reconstituted 100X Renin Red Substrate stock solution (Component A) with Assay Buffer (Component C) at 1:100 as shown in Table 1.

Components	**Volume**
100 X Renin Red Substrate Solution	50 µL
Assay Buffer (Component C)	5 mL
Total volume	5.05 mL

Table 1: Renin Assay Mixture for one 96-well plate (100 assays)

B. Prepare serially diluted Renin standards (0 to 1 µg/mL):

1. Add 12.5 µL of 40 µg /mL Renin Standard (Component B) into 487.5 µL of Assay Buffer (Component C) to get 1 µg /mL Renin standard solution.

2. Take 150 µL of 1 µg/mL Renin standard solution to perform 1:3 serial dilutions to get 300, 100, 30, 10, 3, 1 and 0 ng /mL serially diluted Renin standards.

3. Add Renin standards and/or Renin-containing test samples into a black wall/solid bottom 96-well microplate as described in Tables 2 and 3.

BL	BL	TS	TS	….	….						
Ren 1	Ren 1	….	….	….	….						
Ren 2	Ren 2										
Ren 3	Ren 3										
Ren 4	Ren 4										
Ren 5	Ren 5										
Ren 6	Ren 6										
Ren 7	Ren 7										

Table 2. Layout of Renin standards and test samples in a solid black 96-well microplate.

Note: Ren= Renin Standards, BL=Blank control, TS=test samples.

Renin Standard	Blank Control	Test Sample
Serial Dilutions*: 50 µL	Assay Buffer: 50 µL	50 µL

Table 3. Reagent composition for each well.

**Note 1: Add the serial dilutions of Renin standard from 1 ng/mL to 1000 ng/mL into wells from Ren 1 to Ren 7 in duplicate.*

Note 2: For 384-well plates, use 25 µL/well.

C. Run the enzyme reaction:

1. Pre-incubate the plate at a desired temperature for the enzyme reaction (e.g. 25 °C or 37 °C) for 10-15 min if you are screening Renin inhibitors.

2. Add 50 µL (96-well) or 25 µL (384-well) Renin Red Substrate solution to the standard, sample, and control wells of the assay plate.

3. Incubate the reaction at 37 °C incubator for 30 to 60 minutes.

4. Monitor the fluorescence intensity with a fluorescence plate reader at Ex/Em = 540/590 nm (cut off = 570 mn).

For kinetic reading: Immediately start measuring fluorescence intensity and continuously record data every 5 minutes for 30 to 60 minutes.

For end-point reading: Incubate the reaction at 37°C for 60 minutes or longer, kept from light if possible. And then measure the fluorescence intensity.

7. Data Analysis

The fluorescence in the substrate control well is used as a control, and is subtracted from the values for other wells with the enzyme reactions.

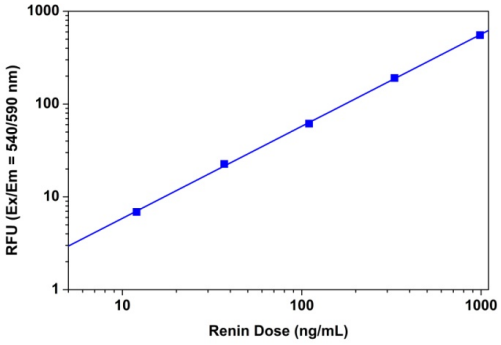


Figure 1. Renin dose response was measured with ab138875 in a 96-well black solid plate using a fluorescence microplate reader. As low as 10 ng /mL Renin was detected with 60 minutes incubation in 37°C

8. Troubleshooting

Problem	Reason	Solution
Assay not working	Assay buffer at wrong temperature	Assay buffer must not be chilled - needs to be at RT
	Protocol step missed	Re-read and follow the protocol exactly
	Plate read at incorrect wavelength	Ensure you are using appropriate reader and filter settings (refer to datasheet)
	Unsuitable microtiter plate for assay	Fluorescence: Black plates (clear bottoms); Luminescence: White plates; Colorimetry: Clear plates. If critical, datasheet will indicate whether to use flat- or U-shaped wells
Unexpected results	Measured at wrong wavelength	Use appropriate reader and filter settings described in datasheet
	Samples contain impeding substances	Troubleshoot and also consider deproteinizing samples
	Unsuitable sample type	Use recommended samples types as listed on the datasheet
	Sample readings are outside linear range	Concentrate/ dilute samples to be in linear range

Problem	Reason	Solution
Samples with inconsistent readings	Unsuitable sample type	Refer to datasheet for details about incompatible samples
	Samples prepared in the wrong buffer	Use the assay buffer provided (or refer to datasheet for instructions)
	Samples not deproteinized (if indicated on datasheet)	Use the ***10kDa spin column (ab93349)*** or ***Deproteinizing sample preparation kit (ab93299)***
	Cell/ tissue samples not sufficiently homogenized	Increase sonication time/ number of strokes with the Dounce homogenizer
	Too many freeze-thaw cycles	Aliquot samples to reduce the number of freeze-thaw cycles
	Samples contain impeding substances	Troubleshoot and also consider deproteinizing samples
	Samples are too old or incorrectly stored	Use freshly made samples and store at recommended temperature until use
Lower/ Higher readings in samples and standards	Not fully thawed kit components	Wait for components to thaw completely and gently mix prior use
	Out-of-date kit or incorrectly stored reagents	Always check expiry date and store kit components as recommended on the datasheet
	Reagents sitting for extended periods on ice	Try to prepare a fresh reaction mix prior to each use
	Incorrect incubation time/ temperature	Refer to datasheet for recommended incubation time and/ or temperature
	Incorrect amounts used	Check pipette is calibrated correctly (always use smallest volume pipette that can pipette entire volume)

Standard curve is not linear	Not fully thawed kit components	Wait for components to thaw completely and gently mix prior use
	Pipetting errors when setting up the standard curve	Try not to pipette too small volumes
	Incorrect pipetting when preparing the reaction mix	Always prepare a master mix
	Air bubbles in wells	Air bubbles will interfere with readings; try to avoid producing air bubbles and always remove bubbles prior to reading plates
	Concentration of standard stock incorrect	Recheck datasheet for recommended concentrations of standard stocks
	Errors in standard curve calculations	Refer to datasheet and re-check the calculations
	Use of other reagents than those provided with the kit	Use fresh components from the same kit

For further technical questions please do not hesitate to contact us by email (technical@abcam.com) or phone (select "*contact us*" on www.abcam.com for the phone number for your region).

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