Amplite® Fluorimetric Renin Assay Kit *Red Fluorescence*

Catalog number: 13530
Unit size: 100 tests

Component	Storage	Amount (Cat No. 13530)
Component A: Renin Red™ Substrate (100X)	Freeze (< -15 °C), Minimize light exposure	1 vial (50 µL)
Component B: Renin Standard	Freeze (< -15 °C), Minimize light exposure	1 vial (40 µg/mL, 25 µL)
Component C: Assay Buffer	Freeze (< -15 °C)	1 bottle (10 mL)

OVERVIEW

Renin is an enzyme that participates in the body's renin-angiotensin system (RAS) that mediates extracellular volume and arterial vasoconstriction. It regulates blood pressure and electrolyte homoeostasis. 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. Renin has been identified to be an attractive target for the treatment of hypertension. The Amplite® Renin Assay Kit provides a convenient assay for high throughput screening of renin inhibitors and renin activity using our proprietary Tide Fluor™ 3 (TF3)/Tide Quencher™ 3 (TQ3) fluorescence resonance energy transfer (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, and the fluorescent signal can be easily monitored by a fluorescence microplate reader. This assay is about fifty fold more sensitive than an EDANS/DABCYL-based assay. With the Amplite® Renin Assay Kit, we have detected as little as 1ng renin in a 100 µL reaction volume. However, the selectivity of the rennin substrate used in the kit has not been thoroughly tested. It may also response to other proteases since peptide-based protease substrates generally have low selectivity.

AT A GLANCE

Protocol Summary

1. Prepare and add Renin standard and test samples (50 µL)
2. Add Renin Red™ substrate working solution (50 µL)
3. Incubate for 30 - 60 min at 37 °C incubator (for end point reading)
4. Monitor fluorescence intensity at Ex/Em = 540/590 nm

Important Note

Thaw all the kit components at room temperature before starting the experiment. Prepare Renin containing biological samples as desired.

KEY PARAMETERS

Fluorescence microplate reader

Cutoff	570 nm
Emission	590 nm
Excitation	540 nm
Recommended plate	Solid black

PREPARATION OF STANDARD SOLUTIONS

For convenience, use the Serial Dilution Planner:
https://www.aatbio.com/tools/serial-dilution/13530

Renin standard

Add 12.5 µL of 40 µg/mL Renin Standard (Component B) into 487.5 µL of Assay Buffer (Component C) to get 1000 ng/mL Renin standard solution (Ren7). Take 150 µL of 1000 ng/mL Renin standard solution to perform 1:3 serial dilutions to get serially diluted Renin standards (Ren6 - Ren1).

PREPARATION OF WORKING SOLUTION

Add 50 µL of Renin Red™ Substrate (Component A) into 5 mL of Assay Buffer (Component C) to make a total volume of 5.05 mL.

Note: The Renin Red™ Substrate should be used promptly.

SAMPLE EXPERIMENTAL PROTOCOL

Table 1. Layout of Renin standards and test samples in a solid black 96-well microplate. Ren = Renin Standards (Ren1-Ren7, 1 to 1000 ng/mL); BL = Blank Control; TS = Test Samples.

BL	BL	TS	TS
Ren1	Ren1	...	...
Ren2	Ren2	...	...
Ren3	Ren3		
Ren4	Ren4		
Ren5	Ren5		
Ren6	Ren6		
Ren7	Ren7		

Table 2. Reagent composition for each well.

Note: The Renin standards are for positive control only, and should not be relied on as a quantitation standard for enzyme activity.

Well	Volume	Reagent
Ren1 - Ren7	50 µL	serial dilution (1 to 1000 ng/mL)
BL	50 µL	Assay Buffer (Component C)
TS	50 µL	sample

1. Prepare Renin containing biological samples as desired.
2. Prepare the Renin standards and/or Renin-containing test samples according to the layout provided in Table 1 and Table 2. For a 384-well plate, use 25 µL of reagent per well instead of 50 µL.
3. 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.
4. Add 50 µL (96-well) or 25 µL (384-well) of Renin Red™ substrate working solution to the Standard sample, control and test wells of the assay plate.
5. Incubate the reaction at 37 °C incubator for 30 to 60 minutes.
6. Monitor the fluorescence intensity with a fluorescence plate reader at Ex/Em = 540/590 nm (cut off = 570 mn).

Note: The selectivity of the renin substrate used in the kit has not been thoroughly tested. It may also respond to other proteases since peptide-based protease substrates generally have low selectivity. One might use a renin-specific inhibitor for its specific test, such as in the presence of a renin-specific inhibitor, hydrolysis of the substrate is only due to the non-specific protease activity. The difference between the total activity and the activity in the presence of renin specific Inhibitor gives the renin activity in the sample.

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.

EXAMPLE DATA ANALYSIS AND FIGURES

The reading (RFU) obtained from the blank standard well is used as a negative control. Subtract this value from the other standards' readings to obtain the baseline corrected values. Then, plot the standards' readings to obtain a standard curve and equation. This equation can be used to calculate Renin samples. We recommend using the Online Linear Regression Calculator which can be found at:

https://www.aatbio.com/tools/linear-logarithmic-semi-log-regression-online-calculator

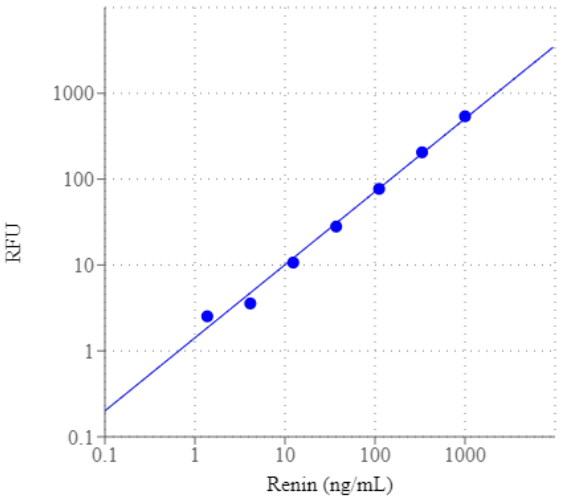

Figure 1. Renin dose response was measured with Amplite® Fluorimetric Renin Assay Kit in a 96-well solid black plate using a Gemini fluorescence microplate reader (Molecular Devices).

DISCLAIMER

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