Amplite™ Fluorimetric Renin Assay Kit **Red Fluorescence**

Ordering Information:	*Storage Conditions:*	*Instrument Platform:*
Product Number: 13530 (100 assays)	Keep at -20 °C and protect from light	Fluorescence microplate readers

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.

The Amplite™ Renin Assay Kit provides a convenient assay for high throughput screening of renin inhibitors and for continuous assay of 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, 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 the Amplite™ Renin Assay Kit, we have detected as little as 1ng renin in a 100 µL reaction volume.

Kit Key Features

Convenient Format:	Include 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.

Kit Components

Components	**Amount**
Component A: Renin Red™ Substrate	1 vial
Component B: Renin Standard	1 vial (40 µg/mL, 25 µL)
Component C: Assay Buffer	1 bottle (10 mL)
Component D: DMSO	200 µL

Assay Protocol for One 96-Well Plate

Brief Summary

Add appropriate controls, or test samples (50 µL) → Add Renin Red™ substrate solution (50 µL) → Incubate for 30-60 min at 37°C incubator (for end point reading) → Monitor fluorescence intensity at Ex/Em = 540/590 nm

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

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1. Prepare Renin containing biological samples as desired.

2. Prepare 100X Renin Red™ Substrate stock solution by adding 50 µL of DMSO (Component D) into the vial of Renin Red™ substrate (Component A).
Note: The stock solution should be used promptly. Any remaining solution should be aliquoted and frozen at -20 °C.

3. Prepare Renin Assay Mixture by dilute reconstituted 100X Renin Red™ Substrate stock solution (from Step 2) with Assay Buffer (Component C) at 1:100 as shown in **Table 1**.

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

Components	**Volume**
100 X Renin Red™ Substrate stock Solution (from Step 2)	50 µL
Assay Buffer (Component C)	5 mL
Total volume	5.05 mL

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

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

4.2 Take 150 µL of 1 µg/mL Renin standard solution (from Step 4.1) to perform 1:3 serial dilutions to get 300, 100, 30, 10, 3, 1 and 0 ng /mL serially diluted Renin standards.

4.3 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

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

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										

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

Table 3. Reagent composition for each well

RENIN Standard	**Blank Control**	**Test Sample**
Serial Dilutions* (50 µL)	Assay Buffer (Component B): 50 µL	50 µL

Note 1: Add the serially diluted Renin standards from 1 ng/mL to 1000 ng/mL into each well from Ren 1 to Ren 7 in duplicate.
Note 2: for 384-well plates, use 25 µL/well.

5. Run the enzyme reaction:

5.1 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.

5.2 Add 50 µL (96-well) or 25 µL (384-well) of Renin Red™ substrate solution (from Step 3) to the sample and control wells of the assay plate.

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

5.4 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.

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.

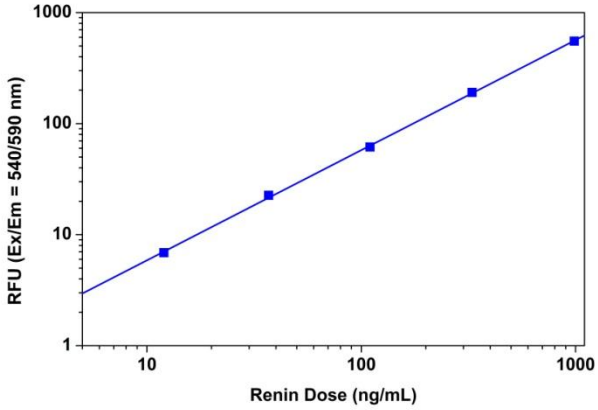

Figure 1. Renin dose response was measured with Amplite™ Fluorimetric Renin Assay Kit in a 96-well black solid plate using a Gemini fluorescence microplate reader (Molecular Devices). As low as 10 ng /mL Renin was detected with 60 minutes incubation in 37°C.

References:

1. De Mello WC, Gerena Y. (2009) Prolonged exposure of cardiac cells to renin plus angiotensinogen reduces intracellular renin in the failing heart. On the role of angiotensin II-AT1 complex internalization. Regul Pept, 155, 139.
2. Friis UG, Madsen K, Svenningsen P, Hansen PB, Gulaveerasingam A, Jorgensen F, Aalkjaer C, Skott O, Jensen BL. (2009) Hypotonicity-induced Renin exocytosis from juxtaglomerular cells requires aquaporin-1 and cyclooxygenase-2. J Am Soc Nephrol, 20, 2154.
3. Schmiedt CW, Hurley KA, Tong X, Rakhmanova VA, Po CL, Hurley DJ. (2009) Measurement of plasma renin concentration in cats by use of a fluorescence resonance energy transfer peptide substrate of renin. Am J Vet Res, 70, 1315.
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5. Wanka H, Kessler N, Ellmer J, Endlich N, Peters BS, Clausmeyer S, Peters J. (2009) Cytosolic renin is targeted to itochondria and induces apoptosis in H9c2 rat cardiomyoblasts. J Cell Mol Med, 13, 2926.