Rat Renin Inhibitor Screening Kit (Fluorometric) (#BN01014)

(Catalog # BN01014; 100 assays; Store at -20°C)

I. Introduction:

Renin (EC 3.4.23.15), also known as an angiotensinogenase, is an enzyme that participates in the renin-angiotensin system (RAS) which mediates extracellular volume (i.e. blood plasma, lymph and interstitial fluid), and arterial vasoconstriction. An over-active renin-angiotensin system leads to vasoconstriction and retention of sodium and water, causing hypertension. Renin inhibitors are widely used for the treatment of hypertension. Assay Genie's Rat Renin inhibitor screening Kit uses a synthetic peptide substrate with a fluorophore (EDANS) at one end and a quencher (DABCYL) at the other end. Renin catalyzes the cleavage of FRET substrate resulting in a product that is detected fluorometrically at Ex/Em = 328/552 nm. In the presence of a Rat Renin inhibitor, the rate of hydrolysis of the substrate is decreased or inhibited. The kit provides a rapid, simple, sensitive, and reliable test suitable for high-throughput screening of rat renin inhibitors. It is also adaptable to a 384-well format.

II. Application:

Studying/characterizing/Screening rat renin inhibitors

III. Kit Contents:

Components	BN01014	Cap Code	Part Number
Renin Assay Buffer	25 ml	WM	BN01014-1
Rat Renin Substrate	200 µl	Red	BN01014-2
Rat Renin Enzyme (lyophilized)	1 vial	Green	BN01014-3
Rat Renin Inhibitor (lyophilized)	1 vial	Blue	BN01014-4

IV. User Supplied Reagents and Equipment:

- 96-well white plate with flat bottom
- Fluorescent microplate reader

V. Storage and Handling:

Store kit at -20°C, protected from light. Avoid repeated freeze/thaw for all non-buffer components. Briefly centrifuge small vials prior to opening. Read the entire protocol before using the kit.

VI. Reagent Preparation & Storage

- **Rat Renin Enzyme:** Dissolve the lyophilized renin in 220 µl Renin Assay Buffer just before use. We recommend that you aliquot the Rat Renin Enzyme solution and store at -80°C. Avoid repeated freeze/thaw cycles. Use within two months. Keep on ice while in use.
- **Rat Renin Inhibitor:** Spin down the contents. Add 110 µl deionized water to make a 25 µM stock solution. Aliquot and store at -80°C.

VII. Renin Inhibitor Screen Assay Protocol:

1. **Enzyme Solution Preparation:** Mix enough reagents for the number of assays to be performed. For each well, prepare 50 µl Renin Enzyme Solution:

   48 µl Renin Assay Buffer
   2 µl Rat Renin Enzyme

   Mix & add 50 µl of the Renin Enzyme Solution into desired wells.

2. **Screen compounds, Inhibitor control and Enzyme Control Preparations:** Make a stock solution of the candidate compounds in an appropriate solvent(s) at 1000x the highest test concentration. Make working solutions of the candidate compounds by diluting the stock solutions to 4x the test concentration in Renin Assay Buffer just before use. For Rat Renin Inhibitor, dilute inhibitor by adding 4 µl Inhibitor stock solution to 21 µl Rat Renin Assay Buffer to prepare a working solution. Add 25 µl of candidate compound working solution, Inhibitor Control (Rat Renin Inhibitor) working solution or Renin Assay Buffer into desired wells containing the Rat Renin Enzyme Solution as candidate screen (S), Inhibitor Control (IC), or Enzyme Control (EC) respectively. Mix well and incubate for 10 min. at 37ºC.

   Note:

   For all inhibitors, make sure that the solvent concentration in the reaction is not greater than 0.1% reaction volume.

3. **Substrate solution preparation:** Make enough substrate solution for the number of wells (sample, Inhibitor Control & Enzyme Control) to be analyzed. For each reaction, prepare a 25 µl substrate solution:

   23 µl Renin Assay Buffer
   2 µl Rat Renin Substrate

   Mix & add 25 µl of the Rat Renin Substrate Solution into each well and mix well.

4. **Measurement**: Measure the fluorescence (Ex/Em = 328/552 nm) in a kinetic mode for 30-60 min at 37ºC. Choose two time points (T1 & T2) in the linear range of the plot and obtain the corresponding values for the fluorescence (RFU1 and RFU2).

5. **Calculation:** Calculate the slope for all samples, including Enzyme Control (EC), by dividing the net ΔRFU (RFU2 - RFU1) values with the time ΔT (T2 - T1). Calculate % Relative Inhibition as follows:

$$\%\ \textbf{Relative Inhibition} = \frac{\textbf{Slope of Enzyme Control} - \textbf{Slope of Sample}}{\textbf{Slope of Enzyme Control}}\ \textbf{x 100}$$

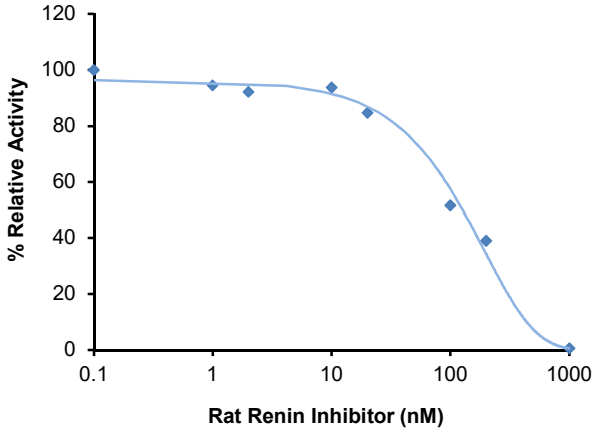

Figure: Inhibition of Rat Renin Enzyme Activity with Rat Renin Inhibitor using kit protocol.

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