Renin Inhibitor Screening Kit (Fluorometric) (#BN01015)

(Catalog # BN01015; 100 assays; Store at -80°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 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 Renin inhibitor, the rate of hydrolysis of the substrate is decreased. The kit provides a rapid, simple, sensitive, and reliable test suitable for high throughput screening of Renin inhibitors also adaptable to a 384 well format.

II. Application:

Screening of renin inhibitors

III. Kit Contents:

Components	BN01015	Cap Code	Part Number
Renin Assay Buffer	25 ml	WM	BN01015-1
Renin Substrate	200 µl	Red	BN01015-2
Active Human Renin (lyophilized) (15 µg)	1 vial	Green	BN01015-3
Inhibitor Control [Aliskiren] (200 µM)	20 µl	Blue	BN01015-4

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. Please read the entire protocol before using the kit.

VI. Reagent Preparation & Storage

- **Active Human Renin:** Dissolve the lyophilized renin in 220 µl Renin Assay Buffer just before use. We recommend that you aliquot the Human Renin Enzyme solution and store at -80°C. Avoid repeated freeze/thaw cycles. Use within two months. Keep on ice while in use.
- **Inhibitor Control (Aliskiren):** Make a stock solution by diluting 1:10 in Renin Assay Buffer just before use by adding 2.5 µl of Aliskiren Inhibitor Control to 22.5 µl of Renin Assay Buffer. Mix well and spin down to collect tube contents.

VII. Renin Inhibitor Screen Assay Protocol:

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

   48 µl Renin Assay Buffer
   2 µl Active Human Renin

   Add 50 µl of the Renin Enzyme Solution to each well.

2. **Screen Compounds, Inhibitor Control and Enzyme Activity Control Preparations:** Make a stock solution of the candidate compounds in appropriate solvent(s) at 1000x the highest test concentration. Make working solutions of the candidate compounds by diluting the stock solutions to 4× the test concentration in Renin Assay Buffer just before use. For all inhibitors, make sure that the solvent concentration in the reaction is not greater than 0.1% reaction volume. For the Aliskiren Inhibitor Control, dilute the stock solution (Step VI), 1:25 in Renin Assay Buffer to prepare a working solution. Add 25 µl of the candidate compound working solution, Inhibitor Control (Aliskiren) working solution or Renin Assay Buffer into wells containing the Renin Enzyme Solution as candidate screen, Inhibitor Control, or Enzyme Activity Control, respectively. Mix well, and incubate for 5 minutes at 37ºC.

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

   23 µl Renin Assay Buffer
   2 µl Renin Substrate

   Add 25 µl of the 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 37°C. 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 Activity Control (EC), by dividing the net ΔRFU (RFU2-RFU1) values with the time ΔT (T2-T1). Calculate % Relative Inhibition as follows:

$$\%\ \textbf{Relative Inhibition} = \frac{(\textbf{Slope of Enzyme Activity Control} - \textbf{Slope of Enzyme Activity with Candidate Screen})}{\textbf{Slope of Enzyme Activity Control}}\ \textbf{x 100}$$

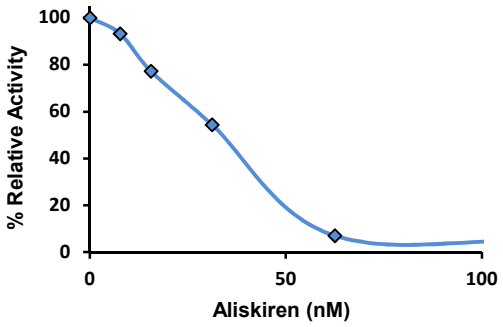

Figure: Inhibition of Renin Enzyme Activity with Inhibitor Aliskiren using kit protocol.

FOR RESEARCH USE ONLY! Not to be used on humans.