ab284512 – Rat Renin Inhibitor Screening Kit (Fluorometric)

For the screening of potential Rat Renin inhibitors.
For research use only - not intended for diagnostic use.

For overview, typical data and additional information please visit:
http://www.abcam.com/ab284512

Storage and Stability

On receipt entire assay kit should be stored at -20°C, protected from light. Kit has a storage time of 1 year from receipt, providing components have not been reconstituted.

Materials Supplied

Item	Quantity	Storage Condition
Renin Assay Buffer	25 mL	-20°C
Rat Renin Substrate	200 µL	-20°C
Rat Renin Enzyme	1 vial	-20°C
Rat Renin Inhibitor	1 vial	-20°C

Materials Required, Not Supplied

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

Reagent Preparation

- Before using the kit, spin the tubes prior to opening.

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.

Assay Protocol

Enzyme Solution preparation:

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

	Enzyme Solution
Renin Assay Buffer	48 µL
Rat Renin Enzyme	2 µL

1. Mix. Add 50 µL of the Renin Enzyme Solution into desired wells.

Screening Compounds, Inhibitor Control, and Blank Control Preparations

1. Make a stock solution of the candidate compounds in an appropriate solvent(s) at 1000x the highest test concentration.
2. Make working solutions of the candidate compounds by diluting the stock solutions to 4x the test concentration in Renin Assay Buffer just before use.
3. 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.

4. 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.
5. Mix well and incubate for 10 minutes at 37°C

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

Substrate Solution Preparation

1. Make enough substrate solution for the number of wells (sample, Inhibitor Control & Enzyme Control) to be analyzed:
2. For each reaction, prepare a 25 µL substrate solution:

	Substrate Solution
Renin Assay Buffer	23 µL
Rat Renin Substrate	2 µL

3. Mix well and add 25 µL of the Rat Renin Substrate Solution into each well. Mix well.

Measurement

Measure the fluorescence (Ex/Em = 328/552 nm) kinetically at 37°C for 30-60 min. Choose two points (T_1 and T_2) in the linear range of the plot and obtain the corresponding fluorescence values (RFU_1 and RFU_2).

Calculation

1. Calculate the slope for all samples, including Enzyme Control (EC), by dividing the net ΔRFU ($RFU_2 - RFU_1$) values by the time Δt ($T_2 - T_1$).
2. Calculate % Relative Inhibition as follows:

$$\% \text{ Relative Inhibition} = \frac{(Slope\ of\ EC - Slope\ of\ S)}{Slope\ of\ EC} \times 100$$

Technical Support

For all technical or commercial enquiries please go to:

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www.abcam.co.jp/contactus (Japan)