Rat Renin Activity Fluorometric Assay Kit (#BN01022)

(Catalog # BN01022; 100 assays; Store at -20°C)

I. Introduction:

Renin (EC 3.4.23.15), also known as 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. In addition to the systemic RAS, locally expressed RAS has been found in the kidney, heart and nervous system. An overactive renin-angiotensin system leads to vasoconstriction and retention of sodium and water, causing hypertension. Renin inhibitors are widely used for the treatment of hypertension. In Assay Genie's Rat Renin Activity Assay Kit, Rat Renin and other proteases hydrolyze a FRET substrate resulting in fluorescence at Ex/Em = 328/552 nm. In the presence of a Rat Renin-Specific Inhibitor, hydrolysis of the substrate is due to only the activity of other proteases. The difference between the total activity and the activity in the presence of Rat Renin-Specific Inhibitor gives the Rat Renin Activity in the sample. This rapid, simple & sensitive kit can detect renin activity as low as 0.12 mU or ~ 0.1µg/ml of recombinant rat renin.

II. Application:

- Detection of Rat Renin Activity

III. Sample Type:

- Crude extracts and purified Recombinant protein
- Tissue lysate

IV. Kit Contents:

Components	BN01022	Cap Code	Part Number
Renin Assay Buffer	25 ml	WM	BN01022-1
Homogenization Buffer	60 ml	NM	BN01022-2
Rat Renin Substrate	200 µl	Red	BN01022-3
Rat Renin (Lyophilized)	1 vial	Green	BN01022-4
Rat Renin Inhibitor (Lyophilized)	1 vial	Blue	BN01022-5
EDANS Standard (100 µM)	100 µl	Yellow	BN01022-6

V. User Supplied Reagents and Equipment:

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

VI. Storage Conditions and Reagent Preparation:

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 performing the experiment.

- **Renin Assay Buffer:** Warm to 37°C before use. Store at -20°C or 4°C
- **Rat Renin:** Dissolve the lyophilized renin in 22 µl Renin Assay Buffer just before use. Aliquot and store at -80°C. Avoid repeated freeze/thaw. Keep on ice while in use. Use within two months.
- **Rat Renin Inhibitor:** Reconstitute with 440 µl deionized water to make a 25 µM stock solution. Aliquot and store at -80°C. Use within two months.

VII. Renin Activity Assay protocol:

1. **Sample Preparation:** Use fresh or frozen (stored at -80ºC) tissue to prepare the tissue extract. Rinse tissue and transfer 100 mg of tissue to a prechilled tube. Add 300 µl cold Homogenization Buffer to the tissue and homogenize tissue on ice thoroughly (polytron homogenizer is recommended). Transfer the contents to a microfuge tube and centrifuge at 16,000 g, 4°C for 10 min. Collect the clarified supernatant in a fresh pre-chilled tube & store on ice. Use immediately to measure renin activity in sample.
2. **EDANS Standard:** Dilute EDANS Standard to 10 µM by adding 10 µl of 100 µM EDANS Standard to 90 µl Renin Assay Buffer. Add 0, 2, 4, 6, 8, & 10 µl of diluted 10 µM EDANS Standard into a series of wells in 96-well plate to generate 0, 20, 40, 60, 80 and 100 pmol/well EDANS Standard. Adjust the volume to 100 µl/well with Renin Assay Buffer.

   Note:

   Dilute the EDANS Standard just before use & discard any unused Standard.
3. **Renin Activity Assay:** Dilute samples 10 times with Homogenization Buffer. For each sample, prepare wells with and without Rat Renin Inhibitor to measure activity in presence of Renin Inhibitor & total protease activity respectively, keeping sample volumes the same. Prepare desired well(s) for sample with & without Renin Inhibitor, Positive Control with & without Renin Inhibitor & Background Control as follows:

	Sample (S)	Sample with Renin Inhibitor (SI)	Positive Control	Positive Control with Renin Inhibitor	Background Control
Sample (1:10 diluted)	2-5 µl	2-5 µl	---	---	---
Rat Renin Inhibitor (25 µM)	---	4 µl	---	4 µl	---
Rat Renin	---	---	2 µl	2 µl	---
Renin Assay Buffer	Make up to 50 µl	Make up to 50 µl	Make up to 50 µl	Make up to 50 µl	50 µl

Preincubate EDANS Standards, samples with & without Renin Inhibitor, Positive control with & without Renin Inhibitor & background control at 37°C for 5 min.

Note: Do not preincubate for longer than 5 min.

4. **Reaction Mix:** Make enough reagents for the number of assays to be performed. For each well, prepare a 50 µl mix containing:

	Reaction Mix
Renin Assay Buffer	48 µl
Renin Substrate	2 µl

Add 50 µl Reaction Mix to each well containing the Positive Control with & without Renin Inhibitor, samples with & without Renin Inhibitor & background control. Mix well.

Note: Warm Assay Buffer to 37°C before use.

5. **Measurement:** Measure the fluorescence (Ex/Em = 328/552 nm) in kinetic mode for 30-60 min. at 37°C. Choose two time points (T_1 & T_2) in the linear range of the plot and obtain the corresponding RFU for sample without renin inhibitor (R_{S1} and R_{S2}) and sample with renin inhibitor (R_{SI1} and R_{SI2}). The EDANS Standard Curve can be read in endpoint mode (i.e., at the end of incubation time).

6. **Calculations:** Subtract 0 Standard reading from all Standard readings. Plot the EDANS Standard Curve. Subtract background control reading from sample readings with and without inhibitor control. Calculate the difference in Protease Activity in the presence and absence of Renin Inhibitor for each sample ΔRFU = (R_{S2} – R_{S1}) – (R_{SI2} – R_{SI1}). Apply the ΔRFU to the Standard Curve to get B pmoles of EDANS liberated by Rat Renin Activity during the reaction time (ΔT = T2 - T1).

$$\textbf{Sample's Renin Activity} = \frac{\mathbf{B}}{\Delta T \times \mu g\ of\ protein} = \textbf{pmol/min/µg = mU/µg}$$

Where: **B** is the EDANS amounts from the Standard Curve (pmol).
ΔT is the reaction time (min.)
µg of protein is the amount of protein/well in µg

Sample Renin Activity can also be expressed as mU/mg of protein.

Unit Definition: One unit of Renin is the amount of enzyme that hydrolyzes the substrate to yield 1.0 nmol of EDANS per min. at 37°C.

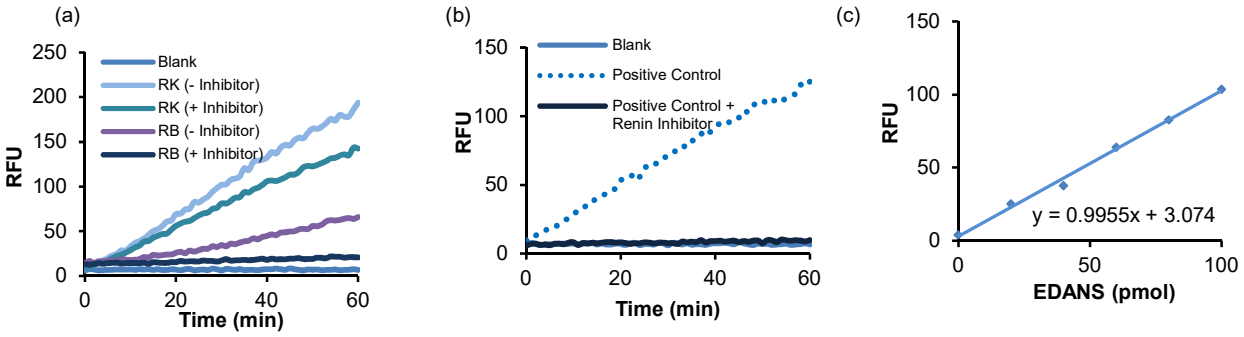

Figure: (a) EDANS Standard Curve, (b) Renin Activity in Positive Control & (c) Renin Activity in Rat Kidney (RK) (28 µg) lysate and Rat Brain (RB) (9.2 µg) lysate with (+) and without (-) Inhibitor. Assays were performed following this kit protocol.

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