

ab284559 – Rat Renin Activity Assay Kit (Fluorometric)

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

For overview, typical data and additional information please visit:

<http://www.abcam.com/ab284559>

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
Homogenization Buffer	60 mL	-20°C
Rat Renin Substrate	200 µl	-20°C
Rat Renin (Lyophilized)	1 Vial	-20°C
Rat Renin Inhibitor (Lyophilized)	1 Vial	-20°C
EDANS Standard (100 µM)	100 µl	-20°C

Materials Required, Not Supplied

These materials are not included in the kit, but will be required to successfully utilize this assay:

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

Reagent Preparation

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.

Assay Protocol

Sample Preparation:

1. Use fresh or frozen (stored at -80°C) tissue to prepare the tissue extract.
2. Rinse tissue and transfer 100 mg of tissue to a prechilled tube.
3. Add 300 µl cold Homogenization Buffer to the tissue and homogenize tissue on ice thoroughly (polytron homogenizer is recommended).
4. Transfer the contents to a microfuge tube and centrifuge at 16,000 g, 4°C for 10 min.
5. Collect the clarified supernatant in a fresh pre-chilled tube & store on ice. Use immediately to measure renin activity in sample.

EDANS Standard:

1. Dilute EDANS Standard to 10 µM by adding 10 µl of 100 µM EDANS Standard to 90 µl Renin Assay Buffer.
2. 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.
3. Adjust the volume to 100 µl/well with Renin Assay Buffer.

ΔNote: Dilute the EDANS Standard just before use & discard any unused Standard.

Renin Activity Assay:

1. Dilute samples 10 times with Homogenization Buffer.
2. 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.
3. 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	-	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

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

ΔNote: Do not preincubate for longer than 5 min.

Reaction Mix:

1. 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

2. 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.

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

Measurement

Measure the fluorescence (Ex/Em = 328/552 nm) in 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 RFU for sample without renin inhibitor (RS1 and RS2) and sample with renin inhibitor (RSI1 and RSI2). The EDANS Standard Curve can be read in endpoint mode (i.e., at the end of incubation time).

Calculation:

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 $\Delta\text{RFU} = (\text{RS2} - \text{RS1}) - (\text{RSI2} - \text{RSI1})$. Apply the ΔRFU to the Standard Curve to get B pmoles of EDANS liberated by Rat Renin Activity during the reaction time ($\Delta\text{T} = \text{T2} - \text{T1}$).

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

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 .

Technical Support

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