ab308273 – Renin Activity Fluorometric Assay Kit

Detects Renin Activity in samples containing renin.
For research use only - not intended for diagnostic use.

For overview, typical data and additional information please visit: abcam website

Storage and Stability

Store kit at -80°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.

Materials Supplied

Item	Quantity	Storage Condition
Renin Assay Buffer	25 ml	---
Renin Substrate (Avoid light)	200 µl	---
Renin-Specific Inhibitor	20 µl	---
Human Renin (Positive Control) (Lyophilized)	1 vial	-80°C
EDANS Standard (100 µM) (Avoid light)	100 µl	---

Materials Required, Not Supplied

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

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

Reagent Preparation

Human Renin (Positive Control): 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.

Renin Activity Assay Protocol

Sample Preparation:

Add 2-48 µl samples into each well and adjust the volume to 50 µl with Renin Assay Buffer. For Background Control (non-specific protease activity), dilute Renin-Specific Inhibitor 10 times by adding 1 µl Renin-Specific Inhibitor to 9 µl Renin Assay Buffer just before use. Add the same amount of samples as used for checking the Renin Activity into desired well(s) and add 2 µl diluted Renin Specific Inhibitor. Adjust the volume to 50 µl with Renin Assay Buffer.

EDANS Standard Curve Preparation

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 to generate 0, 20, 40, 60, 80 and 100 pmol/well EDANS Standard. Adjust the volume to 100 µl with Renin Assay Buffer.

Δ Note: *Dilute the EDANS Standard just before use and discard any unused Standard.*

Positive Control

Add 2-6 µl of Human Renin into desired well(s) and adjust the volume to 50 µl with Renin Assay Buffer.

Reaction Mix:

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

	Reaction Mix
Renin Assay Buffer	48 µl
Renin Substrate	2 µl

Mix well. Add 50 µl of Reaction Mix to each well containing the Positive Control, Samples and Background Controls. Mix well.

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 (RS1 and RS2) and Background (RB1 and RB2). The EDANS Standard Curve can be read in endpoint mode (i.e., at the end of incubation time).

Calculations

Plot the EDANS Standard Curve. Calculate the renin activity of the test sample ΔRFU = (RS2 – RS1) – (RB2 – RB1). Apply the ΔRFU to the Standard Curve to get B pmoles of EDANS liberated during the reaction time (ΔT = T2 - T1).

Sample Renin Activity = B/(ΔT X V) x D = pmol/min./ml = mU/ml

Where: **B** = EDANS amount from the Standard Curve (pmol)
ΔT = reaction time (min.)
V= sample volume added into the reaction well (ml)
D = sample dilution factor

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.

Technical Support

For all technical or commercial enquiries please go to:
www.abcam.com/contactus
www.abcam.cn/contactus (China)
www.abcam.co.jp/contactus (Japan)