CREATIVE BIOMART®
Assay Kit

Renin Activity Fluorometric Assay Kit

Product Information

Cat

Kit-0765

Common Name

Renin

Cat.No. Kit-0765

Product Overview

Rapid, simple & convenient
The assay can detect renin activity as low as 0.75 U/ml.

Description

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. In Renin Activity Assay Kit, Renin and other proteases hydrolyze the FRET substrate resulting in a product that is detected fluorometrically at Ex/Em = 328/552 nm to give total protease activity. In the presence of a Renin-Specific Inhibitor, hydrolysis of the substrate is only due to the non-specific protease activity. The difference between the total activity and the activity in the presence of Renin Specific Inhibitor, gives the Renin Activity in the sample. This rapid, simple & sensitive kit can detect renin activity as low as 0.75 U/ml.

Applications

Detects Renin Activity in samples containing renin.

Usage

For Research Use Only! Not For Use in Humans.

Storage

Store kit at -20°C, protected from light. Avoid repeated freeze/thaw for all non-buffer components.

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Briefly centrifuge small vials prior to opening. Read the entire protocol before using the kit.

Size

100 assays

Kit Components

- Renin Assay Buffer
- Renin Substrate
- Renin-Specific Inhibitor
- Human Renin (Positive Control) (Lyophilized)
- EDANS Standard (100 µM)

Materials Required but Not Supplied

96-well white plate with flat bottom
Fluorescence microplate reader

Target Species

N/A

Detection method Fluorescence (Ex/Em = 328/552 nm)

Compatible Sample Types

Recombinant purified protein

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.

Assay Protocol

1. 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

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Buffer.
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 to generate 0, 20, 40, 60, 80 and 100 pmol/well EDANS Standard. Adjust the volume to 100 μl with Renin Assay Buffer.
Note: Dilute the EDANS Standard just before use & discard any unused Standard.
3. Positive Control: Add 2 μl of Human Renin into desired well(s) and adjust the volume to 50 μl with Renin Assay Buffer.
4. Reaction Mix: Make enough reagents for the number of assays to be performed. For each well, prepare 50 μl mix containing: Renin Assay Buffer 48 μl, Renin Substrate 2 μl
Add 50 μl of Reaction Mix to each well containing the Positive Control, Samples and Background Controls. Mix well.
5. 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).
6. 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*V)
Where: B is the EDANS amount from the Standard Curve (pmol).
ΔT is the reaction time (min.)
V is the sample volume added into the reaction well (ml).
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.