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Product Information

Renin Assay Kit

Catalog Number **MAK157**
Storage Temperature –20 °C

TECHNICAL BULLETIN

Product Description

Renin, also known as angiotensinogenase, is an aspartyl protease that cleaves angiotensinogen to form angiotensin I, the initial and rate-limiting step of the renin-angiotensin system (RAS). This system plays a critical role in the regulation of blood pressure and fluid balance. Increased renin activity can result in hypertension due to increased vasoconstriction and fluid retention. Inhibitors of renin can be used as a treatment for hypertension.

The Renin Assay Kit provides a convenient and simple method for screening renin activity in a variety of samples or for screening renin inhibitors. Renin activity is determined using a proprietary fluorescence resonance energy transfer (FRET) peptide containing a fluorescence quencher. Cleavage of the peptide by renin results in the production of a fluorescent product (λ_{ex} = 540/λ_{em} = 590 nm). This assay is ~50-fold more sensitive than the EDANS/DABCYL-based assay.

Some crude biological samples may contain other protease activity, which cleaves the FRET peptide and interferes with the accurate determination of renin activity. To determine the amount of renin-specific activity, prepare duplicate sample wells with a rennin-specific inhibitor. The difference between the total activity (no inhibitor) and the non-specific activity (with renin inhibitor) is the renin activity of the sample.

Components

The kit is sufficient for 100 assays in 96 well plates.

100× Renin Substrate Catalog Number MAK157A	50 µL
Renin Standard, 40 µg/mL, 25 µL Catalog Number MAK157B	1 vl
Assay Buffer Catalog Number MAK157C	10 mL

Reagents and Equipment Required but Not Provided.

- 96 well flat-bottom plate – It is recommended to use black plates with clear bottoms for fluorometric assays.
- Fluorescence multiwell plate reader

Precautions and Disclaimer

This product is for R&D use only, not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Storage/Stability

The kit is shipped on dry ice and storage at –20 °C, protected from light, is recommended.

Preparation Instructions

Allow all reagents to come to room temperature before use. Briefly centrifuge vials before opening. To maintain reagent integrity, avoid repeated freeze/thaw cycles.

Procedure

All samples and standards should be run in duplicate.

Renin Standards

Add 12.5 µL of the 40 µg/mL Renin Standard to 487.5 µL of Assay Buffer to prepare a 1000 ng/mL (1 µg/mL) Renin Standard Solution. Prepare serial dilutions of the 1000 ng/mL Renin Standard Solution with Assay Buffer to prepare 1,000, 300, 100, 30, 10, 3, 1, and 0 (blank) ng/mL standard solutions. Add 50 µL of the diluted standards to duplicate wells of a 96 well plate.

Note: The renin standards are for positive control only and should not be relied on as a quantitation standard for enzyme activity.

Sample Preparation
Prepare renin-containing biological samples as desired. Add up to 50 µL of sample to wells. Bring samples to a final volume of 50 µL with Assay Buffer.

Note: For unknown samples, it is suggested to test several sample dilutions to ensure the readings are within the linear range of the standard curve.

Assay Reaction
1. Set up the Renin Assay Mixture according to the scheme in Table 1. 50 µL of the Assay Mixture is required for each reaction (well). Any remaining 100× Renin Substrate should be aliquoted and stored at –20 °C to avoid repeated freeze-thaws.

Table 1.
Renin Assay Mixture

Reagent	Volume
100× Renin Substrate	50 µL
Assay Buffer	5 mL

Note: The Renin Assay Mixture is enough for one plate. The amount of Renin Assay Mixture prepared can be scaled if necessary. The Renin Assay Mixture is not stable and best used within 2 hours.

2. If screening renin inhibitors, pre-incubate the plate and the inhibitors for 10–15 minutes at the desired temperature for the enzyme reaction (25 °C or 37 °C).

3. Add 50 µL of the Renin Assay Mixture into each of the sample, standard, and blank wells.

4. Incubate the plate for at the desired temperature for 30–60 minutes taking measurements (λ_{ex} = 540/ λ_{em} = 590 nm, cut off = 570 nm) every 5 minutes. Protect the plate from light during the incubation.

Note: This assay can be adapted for use with 384 well plates. When working with 384 well plates, add 25 µL of standard, sample, and Renin Assay Mixture to each well at the respective steps.

Results
Calculations
The background blank for the assay is the value obtained for the 0 (blank) renin standard. Correct for the background by subtracting the blank value from all readings. Background values can be significant and must be subtracted from all readings.

The relative renin activity can be determined from the standard curve.

Troubleshooting Guide

Problem	Possible Cause	Suggested Solution
Assay not working	Cold Reagents	Assay Buffer must be at room temperature
	Omission of step in procedure	Refer and follow Technical Bulletin precisely
	Plate reader at incorrect wavelength	Check filter settings of instrument
	Type of 96 well plate used	For fluorometric assays, use black plates with clear bottoms
Samples with erratic readings	Cell/Tissue culture samples were incompletely homogenized	Repeat the sample homogenization, increasing the length and extent of homogenization step.
	Samples used after multiple freeze-thaw cycles	Aliquot and freeze samples if needed to use multiple times
	Presence of interfering substance in the sample	If possible, dilute sample further
	Use of old or inappropriately stored samples	Use fresh samples and store correctly until use
	Improperly thawed components	Thaw all components completely and mix gently before use
Lower/higher readings in samples and standards	Allowing the reagents to sit for extended times on ice	Prepare fresh Renin Assay Mixture before each use
	Incorrect incubation times or temperatures	Refer to Technical Bulletin and verify correct incubation times and temperatures
	Incorrect volumes used	Use calibrated pipettes and aliquot correctly
	Use of partially thawed components	Thaw and resuspend all components before preparing the reaction mix
	Pipetting errors in preparation of standards	Avoid pipetting small volumes

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