

ab204723

Renin Inhibitor Screening Assay Kit (Fluorometric)

Instructions for Use

For rapid, sensitive and accurate screening of potential Renin inhibitors.

This product is for research use only and is not intended for diagnostic use.

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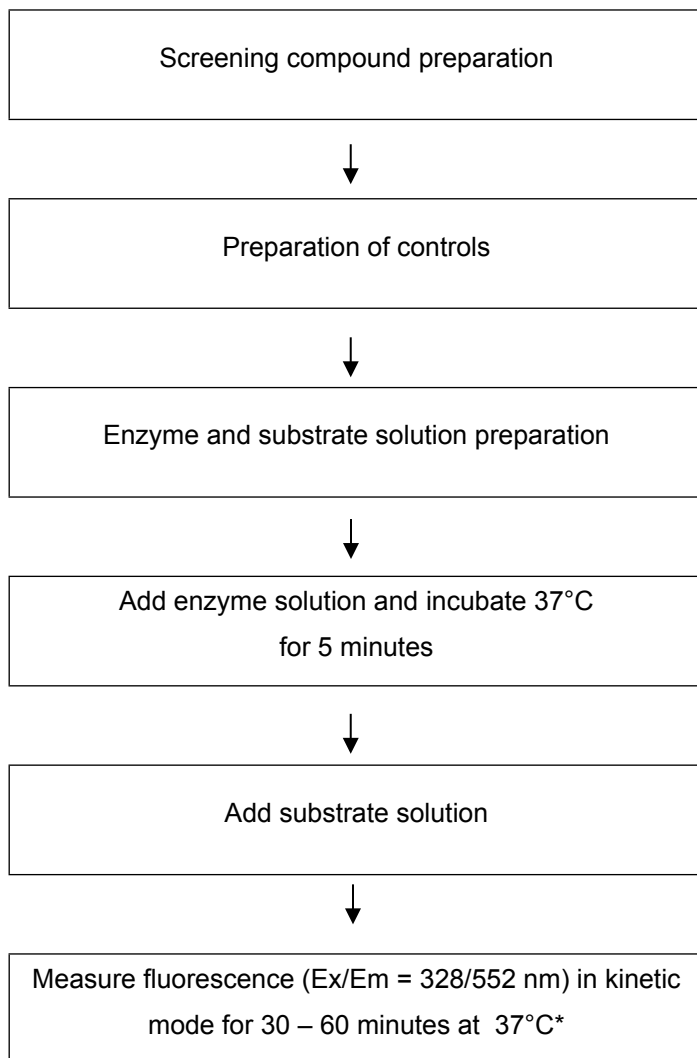
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1. BACKGROUND

Renin Inhibitor Screening Assay Kit (Fluorometric) (ab204723) is an assay based on the use of a synthetic Renin peptide substrate with a fluorophore (EDANS) at one end and a quencher (DABCYL) at the other end. Renin catalyzes the cleavage of FRET substrate resulting in a product that is detected fluorometrically at Ex/Em = 328/552 nm. In the presence of a Renin inhibitor, the rate of hydrolysis of the substrate is decreased. The kit provides a rapid, simple, sensitive, and reliable test suitable for high throughput screening of Renin inhibitors also adaptable to a 384 well format.

Renin (angiotensinogenase, EC 3.4.23.15) 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.

2. ASSAY SUMMARY



**For kinetic mode detection, incubation time given in this summary is for guidance only.*

3. PRECAUTIONS

Please read these instructions carefully prior to beginning the assay.

All kit components have been formulated and quality control tested to function successfully as a kit. Modifications to the kit components or procedures may result in loss of performance.

4. STORAGE AND STABILITY

Store kit at -20°C in the dark immediately upon receipt. Kit has a storage time of 1 year from receipt, providing components have not been reconstituted.

Refer to list of materials supplied for storage conditions of individual components. Observe the storage conditions for individual prepared components in the Materials Supplied section.

Aliquot components in working volumes before storing at the recommended temperature. **Reconstituted components are stable for 2 months.**

5. LIMITATIONS

- Assay kit intended for research use only. Not for use in diagnostic procedures.
- Do not mix or substitute reagents or materials from other kit lots or vendors. Kits are QC tested as a set of components and performance cannot be guaranteed if utilized separately or substituted.

6. MATERIALS SUPPLIED

Item	Amount	Storage Condition (Before Preparation)	Storage Condition (After Preparation)
Renin Assay Buffer	25 mL	-20°C	-20°C
Renin Substrate	200 µL	-20°C	-20°C
Active Human Renin (lyophilized)	15 µg	-20°C	-80°C
Inhibitor Control [Aliskiren] (200 µM)	10 µL	-20°C	-20°C

7. MATERIALS REQUIRED, NOT SUPPLIED

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

- Inhibitor compound of choice
- Pipettes and pipette tips
- Fluorescent microplate reader – equipped with filter
Ex/Em = 328/552 nm
- 96 well plate with clear flat bottom preferably white
- Heat block or water bath

8. TECHNICAL HINTS

- **This kit is sold based on number of tests. A ‘test’ simply refers to a single assay well. The number of wells that contain sample, control or standard will vary by product. Review the protocol completely to confirm this kit meets your requirements. Please contact our Technical Support staff with any questions.**
- Selected components in this kit are supplied in surplus amount to account for additional dilutions, evaporation, or instrumentation settings where higher volumes are required. They should be disposed of in accordance with established safety regulations.
- Keep enzymes, heat labile components and samples on ice during the assay.
- Make sure all buffers and solutions are at room temperature before starting the experiment.
- Avoid foaming or bubbles when mixing or reconstituting components.
- Avoid cross contamination of samples or reagents by changing tips between sample, standard and reagent additions.
- Ensure plates are properly sealed or covered during incubation steps.
- Make sure you have the right type of plate for your detection method of choice.
- Make sure the heat block/water bath and microplate reader are switched on.

9. REAGENT PREPARATION

- Briefly centrifuge small vials at low speed prior to opening.

9.1 **Renin Assay Buffer:**

Ready to use as supplied. Equilibrate to room temperature before use. Store at -20°C.

9.2 **Renin Substrate:**

Ready to use as supplied. Aliquot substrate so that you have enough volume to perform the desired number of assays. Avoid repeated freeze/thaw. Store at -20°C. Keep on ice while in use.

9.3 **Active Human Renin (lyophilized, 15 µg):**

Dissolve the lyophilized renin in 220 µL Renin Assay Buffer just before use. Aliquot renin so that you have enough volume to perform the desired number of assays. Store at -80°C. Avoid repeated freeze/thaw cycles. Use within two months. Keep on ice while in use.

9.4 **Inhibitor Control [Aliskiren] (200µM):**

Ready to use as supplied. Aliquot so that you have enough volume to perform the desired number of assays. Store at -20°C.

Prior to use, make a stock solution by diluting 1:10 in Renin Assay Buffer just before use by adding 1 µL of Aliskiren Inhibitor Control to 9 µL of Renin Assay Buffer. Mix well and spin down to collect tube contents. Keep on ice while in use.

10.SAMPLE PREPARATION

- Always prepare a fresh set of samples and controls for every use.

10.1 Screening Compounds:

- 10.1.1 Dissolve candidate inhibitors at 1000X highest final test concentration into an appropriate solvent.
- 10.1.2 Dilute to 4X the desired test concentration with Renin Assay Buffer.

NOTE: We suggest using different volumes of testing compounds if effective concentration is unknown.

11. ASSAY PROCEDURE and DETECTION

- Equilibrate all materials and prepared reagents to room temperature prior to use.
- It is recommended to assay all controls and samples in duplicate.

11.1 Set up reaction wells:

- Sample wells (S) = 25 μ L test inhibitors.
- Inhibitor Control wells (IC) = 25 μ L Renin inhibitor dilution.
- Enzyme Control wells (EC) = 25 μ L Renin Assay Buffer.
- OPTIONAL: Solvent control (SC) = 25 μ L solvent. **NOTE:** preferred final solvent concentration should not be more than 0.1% reaction volume. If solvent exceeds 0.1%, include solvent control to test the effect on the solvent on enzyme activity.

11.2 Prepare Renin Enzyme Solution:

Prepare 50 μ L of Renin Enzyme Solution for each well:

Component	Enzyme Solution (μ L)
Renin Assay Buffer	48
Active Human Renin	2

Mix sufficient reagents for the number of assays to be performed. Prepare a master mix of the Enzyme Mix to ensure consistency. We recommend the following calculation: X μ L component x (Number reactions + 1).

- 11.3 Add 50 μ L of Renin Enzyme Solution to each well. Mix well.
- 11.4 Incubate at 37°C for 5 minutes.

11.5 Renin Substrate Solution:

Prepare 25 μ L of Renin Substrate Solution for each well:

Component	Substrate Solution (μ L)
Renin Assay Buffer	23
Renin Substrate	2

The table below shows the set up reaction wells:

Component	Sample Well (S) (μL)	Inhibitor Control (IC) (μL)	Enzyme control (EC) (μL)	Solvent Control (SC) (μL)
Test inhibitor compound	25	0	0	0
Renin Assay Buffer	0	24	25	0
Renin Inhibitor Control	0	1	0	0
Solvent test compound	0	0	0	25
Renin Enzyme Solution	50	50	50	50
Renin Substrate Solution	25	25	25	25

- 11.6 Measure fluorescence on a microplate reader at Ex/Em = 328/552 nm in a kinetic mode, every 2 – 3 minutes, for at least 30 – 60 minutes at 37°C.

NOTE: incubation time depends on the Renin activity in samples. Longer incubation times may be required if Renin activity is low.

We recommend measuring the fluorescence in kinetic mode, and choosing two time points (T_1 & T_2) in the linear range and obtain the corresponding values for the fluorescence (RFU1 and RFU2) to calculate the Renin activity of the samples.

12. CALCULATIONS

- For statistical reasons, we recommend each sample should be assayed with a minimum of two replicates (duplicates).
- 12.1 Average the duplicate reading for each test sample compound, Inhibitor Control and Enzyme control.
- 12.2 Choose two time points (T_1 and T_2) in the linear range of the plot and obtain the corresponding values for the fluorescence (RFU₁ and RFU₂).
- 12.3 Calculate the slope for all samples (S), Inhibition Control and Enzyme Control (EC) by dividing the net Δ RFU (RFU₂-RFU₁) value with the time ΔT (T_2 - T_1).
- 12.4 Calculate the % Relative Inhibition as follows:

$$\% \text{ Relative Inhibition} = \frac{\text{Slope of EC} - \text{Slope of S}}{\text{Slope of EC}} \times 100$$

NOTE:

If RFU of SC < RFU of EC = make a higher stock of test inhibitor, or dissolve the inhibitor in lower concentration of the solvent; or use a different solvent.

If RFU of S < RFU of EC = treat as 100% inhibition and further dilute the test inhibitor and repeat the assay.

13. TYPICAL DATA

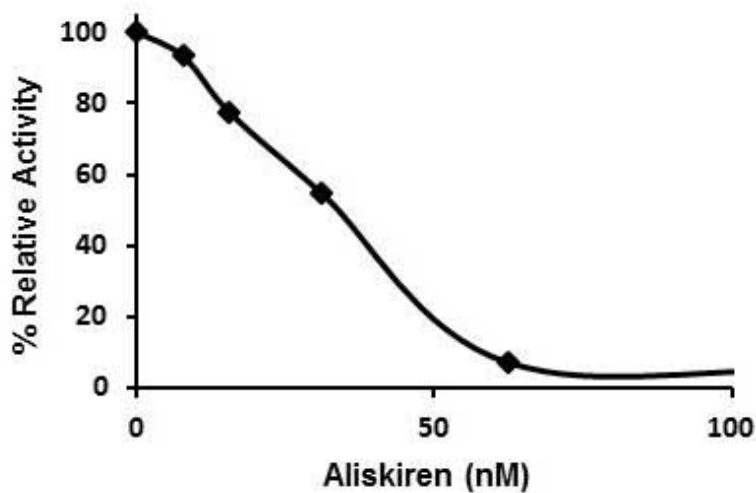


Figure 1. Inhibition of Renin Enzyme Activity with Inhibitor Aliskiren. Assay was performed following kit protocol.

14. QUICK ASSAY PROCEDURE

NOTE: This procedure is provided as a quick reference for experienced users. Follow the detailed procedure when performing the assay for the first time.

- Prepare enzyme mix, substrate mix and get equipment ready. Prepare samples and dissolve test inhibitors in suitable solvent.
- Prepare Renin Enzyme solution for all wells to be set up (50 μL /well)

Component	Enzyme Solution (μL)
Renin Assay Buffer	48
Human Renin Enzyme	2

- Set up plate as follows:

Component	Sample Well (S) (μL)	Solvent control (SC) (μL)	Enzyme Control (EC) (μL)	Inhibitor Control (IC) (μL)
Enzyme Mix	50	50	50	50
Solvent test compound	0	25	0	0
Test Inhibitor Compound	25	0	0	0
Assay Buffer	0	0	25	24
Inhibitor control	0	0	0	1

- Incubate 37°C 5 min.
- Prepare 25 μL of Renin Substrate Mix for each well

Component	Substrate Solution (μL)
Renin Assay Buffer	23
Renin Substrate	2

- Add 25 μL of Renin Substrate Solution to each of S, EC, IC and SC wells.
- Measure plate in a kinetic mode at Ex/Em = 328/552 nm for 30 – 60 minutes at 37°C.

15. TROUBLESHOOTING

Problem	Cause	Solution
Assay not working	Use of ice-cold buffer	Buffers must be at room temperature
	Plate read at incorrect wavelength	Check the wavelength and filter settings of instrument
	Use of a different 96-well plate	Colorimetric: Clear plates Fluorometric: black wells/clear bottom plate
Lower/ Higher readings in samples and Standards	Improperly thawed components	Thaw all components completely and mix gently before use
	Allowing reagents to sit for extended times on ice	Always thaw and prepare fresh reaction mix before use
	Incorrect incubation times or temperatures	Verify correct incubation times and temperatures in protocol
Standard readings do not follow a linear pattern	Pipetting errors in standard or reaction mix	Avoid pipetting small volumes ($< 5 \mu\text{L}$) and prepare a master mix whenever possible
	Air bubbles formed in well	Pipette gently against the wall of the tubes
	Standard stock is at incorrect concentration	Always refer to dilutions on protocol
Unanticipated results	Measured at incorrect wavelength	Check equipment and filter setting
	Samples contain interfering substances	Troubleshoot if it interferes with the kit
	Sample readings above/ below the linear range	Concentrate/ Dilute sample so it is within the linear range

16. FAQ

17. NOTES

Technical Support

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