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ab273373 Angiotensin II Converting Enzyme (ACE2) Inhibitor Screening Kit

View Kit datasheet: https://www.abcam.com/ab273373
(use https://www.abcam.cn/ab273373 for china, or
https://www.abcam.co.jp/ab273373 for Japan)

For the screening and characterization of ACE2 inhibitors.

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

PLEASE NOTE: With the acquisition of BioVision by Abcam, we have made some changes to component names and packaging to better align with our global standards as we work towards environmental-friendly and efficient growth. You are receiving the same high-quality products as always, with no changes to specifications or protocols.

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1. Overview

Angiotensin II Converting Enzyme (ACE2) Inhibitor Screening Kit (ab273373) can be used to screen for potent inhibitors of ACE2 activity, it utilizes the ability of an active ACE2 to cleave a synthetic MCA based peptide substrate to release a free fluorophore. The released MCA can be easily quantified using a fluorescence microplate reader. In the presence of an ACE2 specific inhibitor, the enzyme loses its peptidase activity which results in decrease of fluorescence intensity. This assay kit is simple and can be used to identify and characterize ACE2 inhibitors in a high-throughput format.

2. Protocol Summary

Prepare Enzyme working solution as directed

Prepare Screening Compounds, Inhibitor Control and Enzyme Controls

Incubate for 15 minutes at room temperature

Add ACE2 Substrate Mix

Measure fluorescence (Ex/Em = 320/420 nm) in kinetic mode for 1 hour at room temperature

Calculate the slope for all wells from the linear part of the fluorescence plot

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.
- We understand that, occasionally, experimental protocols might need to be modified to meet unique experimental circumstances. However, we cannot guarantee the performance of the product outside the conditions detailed in this protocol booklet.
- Reagents should be treated as possible mutagens and should be handled with care and disposed of properly. Please review the Safety Datasheet (SDS) provided with the product for information on the specific components.
- Observe good laboratory practices. Gloves, lab coat, and protective eyewear should always be worn. Never pipette by mouth. Do not eat, drink or smoke in the laboratory areas.
- All biological materials should be treated as potentially hazardous and handled as such. They should be disposed of in accordance with established safety procedures.

4. Storage and Stability

Store kit at -20°C in the dark immediately upon receipt.

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.

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	Quantity	Storage temperature (before prep)
Assay Buffer XI/ACE2 Assay Buffer	25 mL	-20°C or +4°C
ACE2 Dilution Buffer	1.5 mL	-20°C or +4°C
ACE2 Enzyme	20 µL	-20°C
ACE2 Substrate	200 µL	-20°C
ACE2 Inhibitor	50 µL	-20°C

7. Materials Required, Not Supplied

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

- Fluorescence microplate reader capable of measuring fluorescence at Ex/Em = 320/420 nm
- 96-well black plate with flat bottom

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 procedures.
- 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.
- Ensure all reagents and solutions are at the appropriate temperature before starting the assay.
- Samples generating values that are greater than the most concentrated standard should be further diluted in the appropriate sample dilution buffer.
- Make sure all necessary equipment is switched on and set at the appropriate temperature.

9. Reagent Preparation

Briefly centrifuge small vials at low speed prior to opening.

9.1 **Assay Buffer XI/ACE2 Assay Buffer:**

Store at -20 °C or 4 °C. Bring to room temperature before use.

9.2 **ACE2 Dilution Buffer:**

Store at -20 °C or 4 °C. Bring to room temperature before use.

9.3 **ACE2 Enzyme:**

Store at -20°C. Thaw before use. Avoid multiple freeze/thaw of the enzyme. Use within 3 months.

9.4 **ACE2 Substrate:**

Ready to use. Store at -20°C. Thaw before use.

9.5 **ACE2 Inhibitor:**

Store at -20°C. Thaw before use. Avoid multiple freeze/thaw of the inhibitor.

10. Assay Procedure

Thaw all reagents thoroughly and mix gently.

10.1 ACE2 Enzyme working solution:

10.1.1 Add 198 µL of ACE2 Dilution Buffer to the ACE2 enzyme vial. The diluted enzyme can be stored at -20°C in aliquots.

10.1.2 For each well (Enzyme Control-EC, Sample-S, Inhibitor Control-IC and Solvent Control-SC), prepare 50 µL of ACE2 Enzyme Working Solution:

Component	Enzyme Control, Sample, Sample Control, Inhibitor control	Background Control
Assay Buffer XI /ACE2 Assay Buffer	48 µL	50 µL
Diluted ACE2 Enzyme Solution	2 µL	-

10.1.3 Mix well and add 50 µL/well into desired wells in a 96-well microtiter plate

10.2 Screening Compounds, Inhibitor Control & Enzyme Control Preparations:

10.2.1 Dissolve candidate inhibitors at 100X highest final test concentration using preferred solvent. Dilute to 10X the desired test concentration with Assay Buffer XI/ACE2 Assay Buffer.

10.2.2 Add 10 µL test inhibitors (Sample) or Assay Buffer XI/ACE2 Assay Buffer (Enzyme Control and Background Control).

10.2.3 Prepare the inhibitor control by adding 50 µl Assay Buffer XI/ACE2 Assay Buffer to the vial containing ACE2 Inhibitor, mix. For Inhibitor Control, add 10 µL ACE2 Inhibitor into ACE2 enzyme containing well(s).

10.2.4 Incubate at room temperature for 15 minutes.

Δ Note: High solvent concentration might affect the enzymatic activity. Prepare parallel well(s) as Solvent Control to test the effect of the solvent on enzyme activity (same as Enzyme Control in presence of final solvent concentration). In case Solvent Control is significantly different from Enzyme Control, use its value in the calculations below.

10.3 ACE2 Substrate Mix:

10.3.1 Prepare enough reagents for the number of assays to be performed. For each well, prepare 40 µl of the Substrate Mix:

Component	Volume to add per well
Assay Buffer XI/ACE2 Assay Buffer	38 µL
ACE2 Substrate	2 µL

10.3.2 Add 40 µL of ACE2 Substrate Mix into Background Control, Enzyme Control/Solvent Control, Inhibitor Control & Sample wells. Mix well.

10.3.3 Measure fluorescence (Ex/Em = 320/420 nm) in kinetic mode for 1 hour at room temperature.

11. Calculations

11.1 Choose two time points (t_1 & t_2) in the linear range of the plot and obtain the corresponding values for the fluorescence (RFU_1 and RFU_2).

11.2 Calculate the slope for all samples, ΔRFU/Δt.

$$Relative\ activity\ (\%) = \frac{\Delta RFU\ of\ Sample}{\Delta RFU\ of\ Enzyme\ Control}\ \ x\ 100$$

Δ Note: In case where Solvent Control is significantly different from Enzyme Control, use its value in the calculation.

12. Typical Data

Typical standard curve – data provided **for demonstration purposes only**. A new standard curve must be generated for each assay performed.

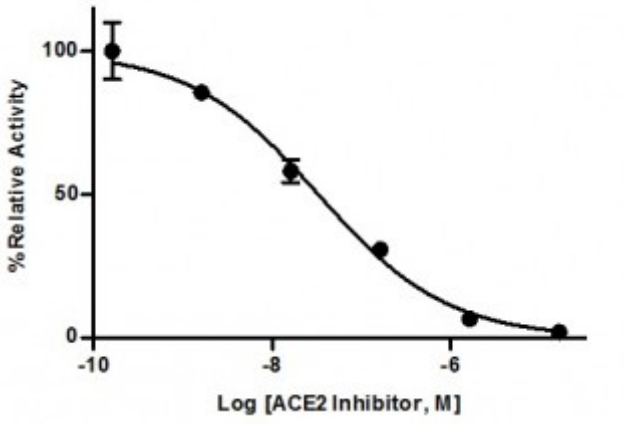

Figure 1. Inhibition of ACE2 activity by ACE2 Inhibitor, IC_{50} = 33.0 ± 0.6 nM (n = 3). Assay was performed following the kit protocol

13. FAQ / Troubleshooting

General troubleshooting points are found at www.abcam.com/assaykitguidelines.

14. Notes

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)