ab283407– Angiotensin Converting Enzyme 1 (ACE1) Assay Kit (Inhibitor Screening, Colorimetric)

For the screening of ACE1 inhibitors.
For research use only - not intended for diagnostic use.

For overview, typical data and additional information please visit:
http://www.abcam.com/ab283407

Storage and Stability

On receipt entire assay kit should be stored at -20°C, protect from light. Upon opening, use kit within 6 months.

Materials Supplied

Item	Quantity	Storage Condition
ACE Assay Buffer	25 mL	-20°C
Colorimetric Substrate	1 vial	-20°C
ACE1 Enzyme II	200 µL	-20°C
ACE1 Inhibitor	100 µL	-20°C
96-Well UV Transparent Plate	1 unit	-20°C

PLEASE NOTE: ACE Assay Buffer was previously labelled as ACE1 Assay Buffer, and Colorimetric Substrate as ACE1 Substrate, and ACE1 Enzyme II as ACE1 Enzyme, and ACE1 Inhibitor as ACE1 Inhibitor Control. The composition has not changed.

Materials Required, Not Supplied

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

– Temperature-controlled plate reader

Reagent Preparation

– Before using the kit, spin the tubes prior to opening.

ACE Assay Buffer: Warm to room temperature (RT) before use. Store at -20°C.

Colorimetric Substrate: Reconstitute with 1.1 mL ACE Assay Buffer. Vortex to dissolve completely. Store at -20°C. Thaw before use.

ACE1 Enzyme II: Aliquot in 10 µL aliquots and store at -20°C. Avoid multiple freeze-thaw cycles of the enzyme. Use within 6 months.

ACE1 Inhibitor: Aliquot and store at -20°C.

96-Well UV Transparent Plate: Ready to use. Bring to room temperature before use.

Assay Protocol

Screening Compounds, Inhibitor Control and Background Control preparations:

- **Test Inhibitor [S]**: Dissolve Test Inhibitor(s) to 100X in a proper solvent. For each Test Inhibitor, dilute to 10X the desired test concentration using ACE Assay Buffer. To determine IC_{50} values for Test Inhibitor(s), prepare several dilutions of the Test Inhibitor(s) in ACE Assay Buffer. Add 25 µL of each dilution into designated well(s).

- **Inhibitor Control [IC]**: Dilute the stock ACE1 Inhibitor to 100 µM Captopril by adding 5 µL of 10 mM Captopril to 495 µL Assay Buffer. Prepare 10 µM working solution of Captopril by adding 50 µL of 100 µM of Captopril and 450 µL Assay Buffer. Add 25 µL of 1 µM Captopril into Inhibitor Control well(s).

Δ Note: *Do not store the diluted Captopril solution. Discard the unused diluted solution.*

Δ Note: *a) Various organic solvents may affect the ACE1 enzymatic activity. We recommend preparing a parallel Solvent Control [SC] well used to at the final well concentration of the solvent used to test Inhibitor(s). If SC slope is significantly different from Enzyme Control [EC], use SC values to determine the effect of the respective Test Inhibitor (see Step 5).*
b) We recommend testing several dilutions of the diluted ACE1 Inhibitor.

- **ACE1 Enzyme Solution Preparation**: Prepare diluted ACE1 Enzyme Solution by adding 2 µL of the stock ACE1 Enzyme II to 38 µL of ACE Assay Buffer. Mix thoroughly and keep on ice.

- **Screening inhibitors:**
  1. Prepare wells containing diluted Test Inhibitor(s) [S], Inhibitor Control [IC], Enzyme Control [EC], Background Control containing no enzyme [BC] and Solvent Control [SC].

	[S]	[IC]	[EC]	[BC]	[SC]
Test Inhibitor	25 µl	---	---	---	---
Inhibitor Control	---	25 µl	---	---	---
ACE Assay Buffer	---	---	25 µl	25 µl	
Solvent Control	---	---	---	---	25 µl

  2. Add 40 µL of diluted ACE1 Enzyme Solution to each well containing [S], [IC], [EC] and [SC] only. Adjust the total volume of each well including [S], [IC], [EC], [SC] and [BC] to 200 µL/well with ACE Assay Buffer.
  3. Mix well and incubate at 37°C for 15-20 minutes, protected from light.

Δ Note: *Do not store diluted ACE1 Enzyme Solution. Discard the unused diluted Enzyme Solution.*

- **Reaction Mix Preparation:**
  1. Mix enough reagents for the number of assays to be performed. For each well, prepare a total of 50 µL Reaction Mix containing:

	Reaction Mix
ACE Assay Buffer	40 µL
Colorimetric Substrate	10 µL

  2. Add 50 µL Reaction Mix to [S], [IC], [EC], [SC] and [BC] wells and mix well.

Measurement

Measure the absorbance immediately at OD 345 nm in kinetic mode for 60 minutes at 37°C. Choose any two time points (t_1 and t_2) in the linear range of the plot and obtain the corresponding absorbance values (OD_1 and OD_2).

Calculation:

1. Calculate the slope for all Test Samples [S], Enzyme Control [EC], Solvent Control [SC] and Background Control [BC] by dividing the net ΔOD (OD_1 and OD_2) values with the time Δt (t_2-t_1).
2. Subtract the Slope of Background Control [BC] from [S], [EC] and [SC]. If [SC] slope is significantly different when compared to [EC], use [SC] values to determine effect of test inhibitor.

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

$$\% \text{ Relative activity} = \frac{slope\ of\ (S)}{slope\ of\ (EC)} \times 100$$

Technical Support

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