ab283372 – Angiotensin Converting Enzyme 1 (ACE1) Assay Kit (Inhibitor Screening, Fluorometric)

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/ab283372

Storage and Stability

Store kit at -20°C, protected from light. Briefly centrifuge small vials prior to opening. Read entire protocol before performing the assay.

Materials Supplied

Item	Quantity	Storage Condition
ACE1 Assay Buffer	20 mL	-20°C
ACE1 Dilution Buffer	1 mL	-20°C
ACE1 Enzyme	50 µl	-20°C
ACE1 Substrate	300 µl	-20°C
ACE1 Inhibitor	100 µl	-20°C

Materials Required, Not Supplied

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

- 96-well black plate with flat bottom
- Multi-well spectrophotometer (ELISA reader)

Reagent Preparation

- Before using the kit, spin the tubes prior to opening. Use within one year.

ACE1 Assay Buffer and ACE1 Dilution Buffer: Store at -20 °C. Bring to room temperature before use.

ACE1 Enzyme: Store at -20°C. Avoid multiple freeze/thaws of the enzyme. Use within 6 months.

ACE1 Substrate: Ready to use. Store at -20°C. Thaw before use.

ACE1 Inhibitor: Ready to use. Store at -20°C. Thaw before use.

Screening Protocol

ACE1 Enzyme Working Solution Preparation:

1. Add 950 µl of ACE1 Dilution Buffer to the ACE1 enzyme vial. The diluted enzyme can be stored at -20°C in aliquots.
2. For each well (Enzyme Control-EC, Sample-S, Inhibitor Control-IC), prepare 50 µl of ACE1 Enzyme Working Solution:

EC, S and IC	Background Control (BC)
40 µl ACE1 Assay Buffer	50 µl ACE1 Assay Buffer
10 µl Diluted ACE1 Enzyme solution	-

3. Mix well and add 50 µl/well into desired wells in a 96-well microtiter plate.

Screening Compounds, Inhibitor Control & Enzyme Control Preparations:

1. Dissolve candidate inhibitors at 100X highest final test concentration using preferred solvent. Dilute to 10X the desired test concentration with ACE1 Assay Buffer.
2. Add 10 µl test inhibitors (Sample, S) or ACE1 Assay Buffer (Enzyme Control or BC).
3. For Inhibitor Control (IC): Add 10 µl ACE1 Inhibitor into ACE1 enzyme containing well(s). Incubate at 37°C for 15 min.

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 EC in presence of final solvent concentration). In case SC is significantly different from EC, use its value in the calculations below.

ACE1 Substrate Mix:

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

Item	Substrate Mix
ACE1 Assay Buffer	37 µl
ACE1 Substrate	3 µl

2. Mix & add 40 µl of ACE1 Substrate Mix into Enzyme Control/Solvent Control, Inhibitor Control, Background control & Sample (S) wells. Mix well.

Measurement

Measure fluorescence (Ex/Em = 330/430 nm) in a kinetic mode for 1-2 hr at 37°C.

Calculation

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). Calculate the slope for all samples, ΔRFU/ΔT.

$$\textbf{\textit{Relative Activity}}\ (\%) = \frac{\Delta\ RFU/\Delta t\ (of\ S)}{\Delta\ RFU/\Delta t\ (of\ EC)} \times\ 100$$

Where
ΔRFU/Δt (of S) – slope calculated for sample wells
ΔRFU/Δt (of EC) – slope calculated for enzyme control wells

Notes: In case SC is significantly different from EC, use its value in the calculations. If all samples and enzyme controls are done at the same time, the equation simplifies to:

$$\textbf{\textit{Relative Activity}}\ (\%) = \frac{\Delta\ RFU\ of\ S}{\Delta\ RFU\ of\ EC} \times\ 100$$

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)