Technical Bulletin

ACE1 Inhibitor Screening Kit (Colorimetric)

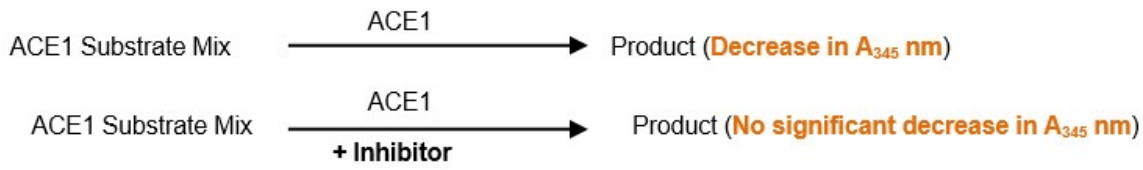
Catalog Number MAK422

Product Description

Angiotensin-I-converting enzyme (ACE) is a peptidyl dipeptidase that catalyzes the conversion of the decapeptide Angiotensin I to the octapeptide Angiotensin II by removing a carboxy-terminal dipeptide. ACE is a key part of the renin angiotensin system that regulates blood pressure. ACE inhibitor inhibits ACE1 enzymatic activity and decreases the production of angiotensin II. As a result, blood vessels are dilated which increases the amount of blood pumped by the heart and lowering the blood pressure. ACE inhibitors are used for treating hypertension, heart failure, stroke, etc. Research has also focused on the discovery of natural products as ACE1 Inhibitors.

The ACE1 Inhibitor Screening Kit can be used to screen potential ACE1 inhibitors. The assay utilizes the ability of an active ACE1 to hydrolyze a synthetic substrate, which results in the decrease in absorbance measured at 345 nm (A_{345}). In the presence of Captopril, an ACE1 specific inhibitor, the ACE1 enzymatic activity is greatly reduced and there is no significant decrease in A_{345} value. The kit provides a rapid, simple and reliable test for high-throughput screening of ACE1 inhibitors.

The kit is suitable for screening and characterizing ACE1 inhibitors.



Components

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

- ACE1 Assay Buffer 25 mL
  Catalog Number MAK422A
- ACE1 Substrate 1 vial
  Catalog Number MAK422B
- ACE1 Enzyme 200 µL
  Catalog Number MAK422C
- ACE1 Inhibitor Control 100 µL
  Catalog Number MAK422D

Reagents and Equipment Required but Not Provided

- Pipetting devices and accessories (including multichannel pipettor)
- 96 well flat-bottom plate suitable for UV spectral measurements. Cell culture or tissue culture treated plates are **not** recommended.
- Temperature-controlled spectrophotometric multi-well plate reader

Precautions and Disclaimer

For Research Use Only. Not for use in diagnostic procedures. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Storage/Stability

The kit is shipped on wet ice. Store kit at -20 °C, protected from light.

Preparation Instructions

Briefly centrifuge small vials prior to opening.

ACE1 Assay Buffer: Ready to use as supplied. Store at 4 °C or -20 °C. Bring to room temperature prior to use.

ACE1 Substrate: Reconstitute vial with 1.1 mL of ACE1 Assay Buffer. Vortex to dissolve completely. Store at -20 °C. Thaw before use.

ACE1 Enzyme: Ready to use as supplied. Aliquot in 10 µL aliquots and store at -20 °C. Avoid multiple freeze-thaw cycles of the enzyme. Use within six months.

ACE1 Inhibitor Control (10 mM Captopril): Ready to use as supplied. Aliquot and store at -20 °C.

Procedure

All samples and standards should be run in duplicate.

Test Inhibitor (S) Preparation

1. Dissolve test inhibitor(s) to 100× in appropriate solvent.
2. Further dilute 10-fold (10×) with ACE1 Assay Buffer. Add 25 µL of each dilution into designated well(s) as Sample (S).
3. Additional wells with serial dilutions of the test inhibitors may be prepared at this time, if desired, containing 25 µL in each candidate well if IC_{50} values need to be estimated.

Inhibitor Control (IC) Preparation

1. Dilute the ACE1 Inhibitor Control (10 mM Captopril) to 100 µM Captopril by adding 5 µL of the 10 mM Captopril to 495 µL of ACE1 Assay Buffer.
2. Prepare a 1 µM working solution of Captopril by adding 5 µL of the 100 µM Captopril from Step 1 to 495 µL of ACE1 Assay Buffer.
3. Add 25 µL of 1 µM Captopril into Inhibitor Control (IC) well(s). Testing several dilutions of the diluted ACE1 Inhibitor Control is recommended.
4. Discard the unused diluted Captopril solutions; do not store.

Solvent Control (SC) Preparation

Various organic solvents may affect the ACE1 enzymatic activity. Prepare parallel Solvent Control (SC) wells by adding 25 µL of solvent at the same concentration as is present in the test inhibitor Sample (S) well(s).

Background Control (BC) and Enzyme Control (EC)

Add 25 µL of ACE1 Assay Buffer to designated well(s).

ACE1 Enzyme Solution Preparation

1. Prepare enough Diluted ACE1 Enzyme Solution for the number of wells to be analyzed. For each well of Test Inhibitor (S), Inhibitor Control (IC), Solvent Control (SC) and Enzyme Control (EC) (but **not** Background Control (BC) wells), prepare 40 µL of Diluted ACE1 Enzyme Solution according to Table 1. Mix well. Discard any unused Diluted ACE1 Enzyme Solution; do not store.

Table 1.
Diluted ACE1 Enzyme Solution

Reagent	Volume
ACE1 Enzyme	2 µL
ACE1 Assay Buffer	38 µL

2. Add 40 µL of Diluted ACE1 Enzyme Solution to each well Test Inhibitor (S), Inhibitor Control (IC), Solvent Control (SC), and Enzyme Control (EC). Do **not** add Diluted ACE1 Enzyme Solution to Background Control (BC) wells.
3. Adjust the total volume of all wells to 200 µL/well with ACE1 Assay Buffer.
4. Mix well and incubate at 37 °C for 15-20 minutes, protected from light.

Reaction Mix

1. Mix enough reagents for the number of assays to be performed. For each well, prepare 50 µL of Reaction Mix according to Table 2. Mix well.

Table 2.
Preparation of Reaction Mix

Reagent	Volume
ACE1 Assay Buffer	40 µL
ACE1 Substrate Mix	10 µL

2. Add 50 µL of Reaction Mix to each well, mix well.

Measurement

Measure the absorbance immediately at 345 nm (A_{345}) in kinetic mode for 60 minutes at 37 °C. Choose two time points (T_1 and T_2) in the linear range of the plot and obtain the corresponding absorbance values ($A_{345\ 1}$ and $A_{345\ 2}$).

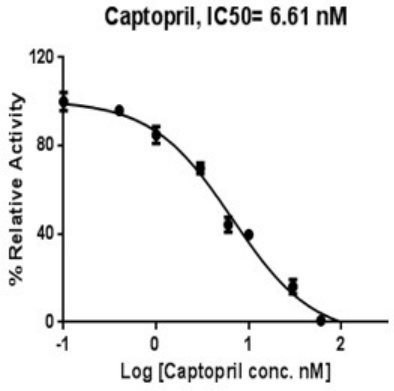
Results

1. Calculate the slope for all samples [(S), (IC), (SC), (EC), and (BC)]by dividing the net A_{345} ($A_{345\ 2}$ - $A_{345\ 1}$) values by the time ΔT (T_2 - T_1).
2. Subtract the Background Control (BC) slope from the slopes of the Test Inhibitor (S), Inhibitor Control (IC), Solvent Control (SC), and Enzyme Control (EC). If Solvent Control (SC) slope is significantly different than the Enyzme Control (EC) slope, use (SC) values to determine the inhibition effect of the solvent and subtract from the Test Inhibitor result.
3. Calculate % Relative Inhibition as follows:

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

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

Figure 1.
Inhibition of ACE1 activity by Captopril. IC_{50} was calculated to be 6.61 nM. Assay was performed following the kit protocol.



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