Technical Bulletin

ACE1 Colorimetric Activity Assay Kit

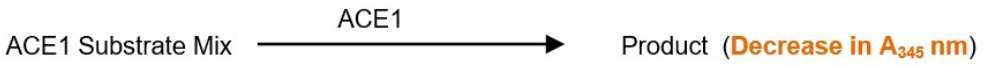
Catalog Number MAK419

Product Description

Angiotensin-I-converting enzyme (ACE1) is a peptidyl dipeptidase that catalyzes the conversion of decapeptide angiotensin I to octapeptide angiotensin II, by removing a carboxy-terminal dipeptide. ACE is a key part of the renin angiotensin system (RAS) that regulates blood pressure. Increased ACE activity leads to an increase in the angiotensin II (Ang II) levels. This may increase the risk of developing neurodegenerative diseases including Parkinson, Alzheimer, Huntington, Multiple Sclerosis etc. Elevated levels of Ang II mediate cardiac remodeling (cardiac hypertrophy and fibrosis), which is a hallmark in heart failure. Research has shown that ACE levels in serum samples is a good predictor of Type 2 diabetes pathologies.

The ACE1 Activity Assay Kit can be used to detect its activity in biological samples. This kit utilizes the ability of an active ACE1 to hydrolyze a synthetic substrate, which results in the decrease in optical density at A_{345} nm. The assay kit provides a rapid, simple and sensitive method to detect ACE1 activity as low as 40 mU.

The kit is suitable for the measurement of ACE1 activity in plasma or serum samples.



Components

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

- ACE1 Assay Buffer — 25 mL
  Catalog Number MAK419A
- ACE1 Substrate — 1 vial
  Catalog Number MAK419B
- ACE1 Enzyme — 50 µL
  Catalog Number MAK419C

Reagents and Equipment Required but Not Provided

- Pipetting devices and accessories (including multichannel pipettor)
- 96-well clear flat-bottom plate suitable for UV readings. Cell culture or tissue culture treated plates are **not** recommended.
- Spectrophotometric multiwell 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: Warm to room temperature prior to use. Store at 2-8 °C.

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

ACE1 Enzyme: Aliquot in 10 mL aliquots and store at -20 °C. Avoid multiple freeze/thaw cycles. Use within six months.

Procedure

All samples and standards should be run in duplicate.

ACE1 Enzyme Working Solution Preparation

Prepare a 20-fold dilution of the ACE1 Enzyme by adding 2 mL of ACE1 stock Enzyme to 38 mL of ACE1 Assay Buffer. Mix thoroughly and keep on ice. **Do not store** the diluted ACE1 Enzyme Solution. Discard any unused solution.

Experimental Design

Plasma or serum samples (fresh or thawed) can be used directly in the assay.

1. Add 30 mL of plasma or serum into well(s) designated as Sample (S) in a 96-well clear bottom UV Plate.
2. For Blank (B), add 50 mL of ACE1 Assay Buffer into designated well(s).
3. For Positive Control (PC), add 40 mL of the Diluted ACE1 Enzyme Solution into designated well(s).
4. Adjust the total volume of Sample(s), Blank and Positive Control to 200 mL/well with ACE1 Assay Buffer.
5. Incubate at 37 °C for 10 minutes.

Diluted ACE1 Substrate

1. Prepare a 5-fold dilution of the ACE1 Substrate with Assay Buffer (50 mL of ACE1 Substrate plus 200 mL of ACE1 Assay Buffer). Prepare enough substrate for the number of assays to be performed. Mix well. Diluted ACE1 Substrate can be stored at -20 °C and used within three months.
2. Add 50 mL of Diluted ACE1 Substrate from Step 1 into each Sample (S), Blank (B), and Positive Control (PC) wells. Mix well and remove any air bubbles.

Measurement

Measure absorbance in kinetic mode at 345 nm (A_{345}) for 60 minutes at 37 °C. Samples having low ACE1 activity can be measured for 1.5 to 2 hours.

Results

1. Take the absorbance results at 345 nm (A_{T1} and A_{T2}) at two time points (T_1 and T_2) respectively in the linear range. There should be at least two readings between the A_{T1} and A_{T2} readings, and the difference between T_1 and T_2 (ΔT) must be at least 1 minute. Note that the change in absorbance (ΔA) will be negative (A1 ≥ A2).

2. Calculate the absolute change in A_{345} absorbance for Sample (S) and Blank (B).

   For Sample (S), $\Delta A_S = A_{T2\ Sample} - A_{T1\ Sample}$

   For Blank (B), $\Delta A_B = A_{T2\ Blank} - A_{T1\ Blank}$

3. To determine the activity of ACE1, use the following equation:

$$\text{ACE1 Activity (U/mL)} = \frac{([|\Delta A_S| / \Delta T] - [|\Delta A_B| / \Delta T]) \times 0.25 \times D}{0.34 \times V}$$

where:

$|\Delta A|$ = Absolute value difference between A_{T2} and A_{T1} at 345 nm for Sample (S) and Blank (B)

ΔT = Difference between T_2 and T_1 (in minutes)

0.25 = Reaction volume (mL)

D = Sample Dilution factor (if applicable; D = for undiluted Samples)

0.34 = Millimolar extinction coefficient of ACE1 Substrate

V = Enzyme/Sample volume (mL)

Figure 1.
Kinetic activity curves of ACE1 activity in Serum (30 mL) and Plasma (30 mL) Samples. Note: Curves were plotted after normalizing the raw values with the maximum absorbance value obtained from each sample.

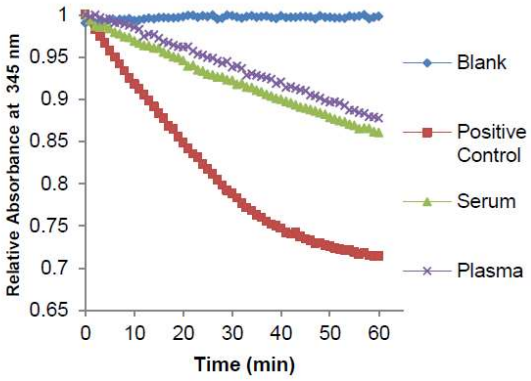
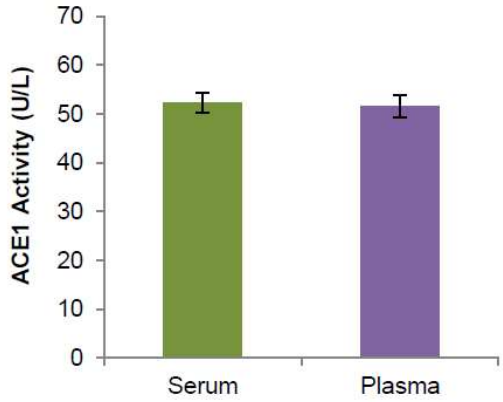


Figure 2.
ACE1 activity in human Serum and Plasma Samples (30 mL). Assays were performed following the kit protocol.



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