Technical Bulletin

Angiotensin I Converting Enzyme (ACE) Activity Assay Kit (Fluorometric)

Sufficient for 200 fluorometric tests

CS0002

Product Description

Angiotensin Converting Enzyme (ACE) is a key component in the renin-angiotensin system (RAS), which participates in the regulation of blood pressure, electrolyte balance, and vascular remodeling.[1,2] ACE is located mainly in the capillaries of the lungs, but can also be found in endothelial and kidney epithelial cells.[3] This hormone system also regulates body fluids volume. Renin produces angiotensin I by cleaving several amino acids from angiotensinogen. ACE then hydrolyzes angiotensin I to yield active angiotensin II. Angiotensin II also stimulates the adrenal cortex to secrete aldosterone, which also causes an increase in blood pressure by stimulating sodium reabsorption by the kidney.[1] Since ACE exhibits critical functions in RAS regulation, ACE inhibition has become an area of research focus in such conditions as hypertension, heart failure, and diabetic nephropathy.[4]

This kit provides a simple, quick, sensitive, and direct procedure to measure ACE levels in various samples, such as serum and plasma, as well as to screen for ACE inhibitors. The assay is based on the cleavage of a synthetic fluorogenic peptide. The measured fluorescence is directly proportional to the ACE activity present. The reaction is summarized as follows:

$$\text{ACE fluorogenic substrate} \xrightarrow{\text{ACE}} \text{Cleaved ACE substrate + fluorescent product } (\lambda_{ex}\ 320\ \text{nm} / \lambda_{em}\ 405\ \text{nm})$$

The ACE Positive Control in the kit can be used to screen for ACE inhibitors. This kit does not require weighing, or mixing of multiple reagents. This kit has a linear range of 1.5-110 mU of ACE activity.

Unit definition: One unit of ACE is defined as the amount of enzyme that releases 1 nmol of fluorescent product from the substrate, in 1 minute, under the assay conditions, at 37 °C.

Precautions and Disclaimer

For R&D use only. Not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Components

This kit contains sufficient reagents for 200 tests**.**

Component	Component Number	Amount	Cap Color/ Container Information
Assay Buffer	CS0002A	30 mL	White cap/ bottle
ACE Positive Control	CS0002B	50 µL	Red cap/ vial
Substrate*	CS0002C	100 µL	Brown vial
Standard*	CS0002D	100 µL	Brown vial

Component Information

- Assay Buffer (CS0002A): Ready-to-use.
- ACE Positive Control (CS0002B): Supplied as a 50 µL solution. Dilute 500-fold in Assay Buffer.
- Substrate (CS0002C): Supplied as a 100× solution. Protect from light. Make sure the solution fully melts prior to use. **Do not place on ice**. Dilute the Substrate 100-fold in Assay Buffer.
- Standard (CS0002D): Supplied as a 1 mM solution. Protect from light. Make sure that the solution fully melts prior to use. **Do not place on ice**. Dilute the Standard 10-fold in Assay Buffer to a final concentration of 100 µM (see Procedure).

Equipment Required, But Not Provided

- 96-well flat-bottom black plates
- Fluorescence multiwell plate reader (λ_{ex} 320 nm / λ_{em} 405 nm)

Storage/Stability

The kit is shipped on dry ice. Upon receipt, store all components at –20 °C, protected from light. Upon thawing, the Assay Buffer can be stored at 2-8 °C. The unopened kit is stable for 2 years as supplied.

Preparation Instructions

1. Thaw all components, and equilibrate to room temperature, before use.
2. Briefly centrifuge vials before opening.
3. Prepare all the specified dilutions immediately prior to performing the assay.
4. Unused components should be stored at –20 °C.
5. Unused diluted material should be discarded.

Procedure

- All samples and standards should be run in duplicate.
- For convenience, an Excel-based calculation sheet is available on the Product Detail Page. Use this sheet to calculate the amounts of reagents required, as well as to calculate the test results.

Sample preparation

1. Tissue culture extracts should be prepared using an appropriate lysis buffer. The use of CelLytic™ M (Cat. No. C2978) is recommended for mammalian cells.
2. Homogenize cells using a Dounce homogenizer. Remove any insoluble material by centrifugation.
3. Serum and plasma samples should be diluted to the appropriate concentration using the Assay Buffer. Best results are obtained when samples are freshly prepared. If this is not feasible, samples should be stored at 2-8 °C and used within 24 hours.

   Note: If desired, the ACE Positive Control can be included, either as a positive control, and/or to screen for ACE inhibitors.
4. Dilute the ACE Positive Control 500-fold in Assay Buffer. As a control, specific inhibitors of ACE can be included (such as 50 µM lisinopril, Cat. No. PHR1143).
5. All samples should be in a final volume of 50 µL. **Note**: Always include a Sample Blank, where the sample is replaced with 50 µL of Assay Buffer.

Standards

1. Dilute the Standard 10-fold to a final concentration of 100 µM: 10 µL of the Standard stock solution with 90 µL of Assay Buffer.
2. Add 0, 1, 2, 3, 4, 5, 6, and 8 µL of the diluted standard solution into a 96-well plate, to generate 0 (blank), 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7 and 0.8 nmol/well standards.
3. Bring the final volume to 100 µL with Assay Buffer. See Table 1:

Table 1. Standard curve preparation per well

10-fold Diluted Standard volume*	Assay Buffer volume*	Amount of Standard per well (nmol)*
0 µL (Standard Blank)	100 µL	0
1 µL	99 µL	0.1
2 µL	98 µL	0.2
3 µL	97 µL	0.3
4 µL	96 µL	0.4
5 µL	95 µL	0.5
6 µL	94 µL	0.6
8 µL	92 µL	0.8

* Work in duplicate

Substrate

- Dilute the Substrate 100-fold in Assay Buffer. A volume of 50 µL is required per each reaction.
- Note: **Do not add the Substrate to the Standard wells**.

Assay reaction

Note: This assay is based on an enzyme-catalyzed kinetic reaction. Addition of Substrate should be **quick**. Measurement should be **immediate**. Use of a multichannel pipette is recommended.

1. Set the fluorescence multiwell plate reader to excitation at 320 nm and emission at 405 nm.
2. Bring the 96-well plate and all the reagents to 37 °C for 5 minutes.
3. Initiate the reaction by adding 50 µL of the diluted Substrate to the sample wells, for a final reaction volume of 100 µL/well. Immediately read the fluorescence in kinetic mode in 5 cycles for 5 minutes.

Results

Calculations

1. An Excel-based calculation sheet is available at the Product Detail Page. **Use this sheet to calculate the test results**.
2. Subtract the Standard Blank value (no Standard) from all standard values.
3. Subtract the Sample Blank value (no Sample) from all sample values.
4. Construct a standard curve:
   - Plot the fluorescence measured for each standard (in RFU) against the amount of the standard (in nmol).
   - Determine the linear regression equation from all standard readings.
5. Construct a sample kinetic curve:
   - Plot the fluorescence measured for each sample (in RFU) against time (in minutes).
   - Determine the linear regression equation for each sample.
6. Use the standard curve linear regression slope and the sample kinetic curve linear regression slope to transform the values of the samples from RFU/min to nmol/min (units):
   - Sample enzymatic activity (nmol/min) = $[Slope_{Sample}]/[Slope_{Std}] \times DF$
   - Where:
     - $Slope_{Std}$ = Slope of blank-subtracted standard curve (RFU/nmol)
     - DF = Dilution factor (if the sample is not diluted, the DF value is 1)
     - $Slope_{Sample}$ = Slope of blank-subtracted sample curve (RFU/min)

Calculation example

$Slope_{Std}$ = 50,000 RFU/nmol (from standard curve)

$Slope_{Sample}$ = 5,000 RFU/min (from sample kinetic curve)

Sample enzymatic activity =
(5,000 RFU/min) / (50,000 RFU/nmol) =
0.1 nmol/min = 0.1 units = 100 mU

References

1. Alvarenga, E.C. *et al.*, *PLoS ONE*, **11(12)**, e0165371 (2016).
2. Skeggs, L.T. *et al.*, *J. Exp. Med.*, **103(3)**, 295-299 (1956).
3. Chamsi-Pasha, M.A. *et al.*, *Curr. Heart Fail. Rep.*, **11(1)**, 58-63 (2014).
4. Zhang, C. *et al.*, *J. Phys. Chem. B*, **117(22)**, 6635- 6645 (2013).

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