Angiotensin I Converting Enzyme 2 (ACE2) Assay Kit

Catalog No.: abx294409

Size: 96 tests

Detection Range: 0.028 U/L – 10.47 U/L

Sensitivity: 0.028 U/L

Storage: Store all components at -20°C. Store the Substrate and Standard in the dark.

Application: For detection and quantification of Angiotensin I Converting Enzyme 2 (ACE2) activity in serum, plasma, animal tissue homogenates and cell samples.

Introduction

Angiotensin I Converting Enzyme 2 (ACE2) is an enzyme that catalyses the cleavage of angiotensin I into angiotensin 1-9 and angiotensin II into the vasodialator angiotensisn 1-7. ACE2 can either be found attached to the membrane of cells (mACE2) or in a soluble form (sACE2). Both membrane-bound and soluble forms of ACE2 are important components of the renin-angiotensin-aldosterone system (RAAS). The membrane bound form of ACE2 (mACE2) also serves as the entry point into cells for some coronaviruses.

Abbexa's Angiotensin I Converting Enzyme 2 (ACE2) Assay Kit is a quick, convenient, and sensitive method for measuring and calculating Angiotensin I Converting Enzyme 2 (ACE2) activity. ACE2 catalyzes the decomposition of substrates which releases fluorescent products. The fluorescence value is directly proportional to the ACE2 activity in the sample which can therefore be measured using a fluorescent microplate reader at Ex 325 nm / Em 395 nm.

Kit components

1. 96-well Black Microplate
2. Substrate: 0.2 ml
3. Buffer Solution: 50 ml
4. Extraction Solution: 2 × 40 ml
5. Standard: 0.6 ml
6. Plate sealer: 2

Materials required but not provided

1. Flourescent Microplate reader (Excitation 325 nm, Emission 395 nm)
2. Double-distilled water
3. Normal saline (0.9 % NaCl)
4. Multichannel pipette, pipette and pipette tips
5. 1.5 ml microcentrifuge tubes
6. Centrifuge
7. Incubator
8. Mechanical homogenizer
9. Ultrasonicator

Protocol

A. Preparation of samples and reagents

1. Samples

Isolate the test samples soon after collecting and analyze immediately or aliquot and store at -20°C or -80°C for long-term storage. Avoid multiple freeze-thaw cycles.

The following sample preparation methods are intended as a guide and may be adjusted as required depending on the specific samples used.

- **Serum and Plasma:** Samples can be tested directly. If the samples are not clear, centrifuge at 10,000 × g at 4°C for 10 minutes, then take the supernatant for detection.
- **Tissue Homogenates:** Carefully weigh 20 mg of tissue and wash in cold normal saline (0.9 % NaCl). Add tissue into 180 µl of Extraction Solution and homogenize manually, using a mechanical homogenizer, at 4°C. Centrifuge at 10,000 × g for 10 minutes at 4°C, then carefully take the supernatant and keep on ice for detection.
- **Cell Adherent or Suspension Cultures:** Harvest 1 × 10^6 cells and wash with normal saline (0.9 % NaCl). Add cells into 200 µl of Extraction Solution, and homogenize by ultrasonication at 4°C. Centrifuge at 10,000 × g for 10 minutes at 4°C, then carefully take the supernatant and keep on ice for detection.

  Note: To calculate ACE2 activity in tissue homogenates and cell samples using the formulae in section **C. Calculation of Results**, the total protein concentration of the supernatant must be determined separately **(abx097193)**.

It is recommended to carry out a preliminary experiment to determine the optimal dilution factor of samples before carrying out the formal experiment. Dilute samples into different concentrations with Buffer Solution, then carry out the assay procedure. The recommended dilution factor for different samples is as follows (for reference only):

Sample Type	Dilution Factor
10 % Mouse kidney tissue homogenates	2 – 8
10 % Mouse lung tissue homogenates	1 – 5
10% Mouse heart tissue homogenates	1
10 % Mouse liver tissue homogenates	1
1 × 10^6 HEK 293 cells	1
1 × 10^6 HL-60 cells	1 – 2
1 × 10^6 HeLa cells	1 – 2

Note:

- Fresh samples or recently obtained samples are recommended to prevent degradation and denaturalization that may lead to erroneous results.

- Lysis buffers may interfere with the kit. It is therefore recommended to use mechanical lysis methods for cell lysates and tissue homogenates.

2. Reagents

- **Substrate Working Solution:** Prepare enough Substrate Working Solution for the amount of wells used. Dilute the Substrate with Buffer Solution in a ratio of 1 : 200 (i.e. add 1 ml of Substrate to 200 ml of Buffer Solution to prepare 201 ml of Substrate Working Solution). Store at 4°C and use within 24 hours.
- **Standard (100 µmol/L) Solution:** Add 20 µl of Standard to 1980 µl of Buffer Solution to prepare 2000 µl of Standard (100 µmol/L) Solution. Store at 4°C and use within 48 hours.
- **Standards:** Label 7 tubes with 100 µmol/L, 80 µmol/L, 70 µmol/L, 60 µmol/L, 50 µmol/L, 40 µmol/L, and 20 µmol/L. Add 50 µl, 40 µl, 35 µl, 30 µl, 25 µl, 20 µl, and 10 µl of Standard (100 µmol/L) to the 100 µmol/L, 80 µmol/L, 70 µmol/L, 60 µmol/L, 50 µmol/L, 40 µmol/L, and 20 µmol/L tubes respectively, followed by 0 µl, 10 µl, 15 µl, 20 µl, 25 µl, 30 µl, and 40 µl of double-distilled water, to prepare Standard Dilutions with concentrations 100 µmol/L, 80 µmol/L, 70 µmol/L, 60 µmol/L, 50 µmol/L, 40 µmol/L, and 20 µmol/L. These volumes are summarized in the following table:

Standard Dilution (µmol/L)	**100**	**80**	**70**	**60**	**50**	**40**	**20**
100 µmol/L Standard (µl)	50	40	35	30	25	20	10
Buffer Solution (µl)	0	10	15	20	25	30	40

For the blank, or 0 µmol/L standard, use pure Buffer Solution. The volume of each standard will be 50 µl.

Note:

- Allow all reagents to equilibrate to room temperature before use.
- When adding the Substrate Working Solution, it is recommended to use a multichannel pipette if the number of samples is large. The reaction will start immediately after adding the substrate.
- Ensure to mix the prepared standard dilutions thoroughly.
- When adding the samples to the microplate, tap the plate gently to mix.

B. Assay Procedure

Pre-heat the incubator and ensure it has reached a stable temperature before use.

1. Assign and record microplate positions for each standard and sample. *It is strongly recommended to prepare all the wells in duplicate.*
2. Add 10 µl of sample to each sample well, tap the plate gently to mix thoroughly.

3. Add 10 µl of prepared standard dilutions to the corresponding standard wells.
4. Add 90 µl of Buffer Solution to the standard wells
5. Add 90 µl of Substrate Working Solution to the sample wells
6. Measure the fluorescence values of each well at Ex 325 nm / Em 395 nm. Record these values as F_1. For example, $F_{1\ Standard}$ and $F_{1\ Sample}$.
7. Incubate at 37°C for 10 minutes.
8. Measure the fluorescence values of each well at Ex 325 nm / Em 395 nm. Record these values as F_2. For example, $F_{2\ Standard}$ and $F_{2\ Sample}$.

C. Calculation of Results

Plot the standard curve, using the F_2 values of the standard dilutions (adjusted for the blank) on the y-axis, and their respective concentrations on the x-axis. The curve should form a straight line, described by the formula $y = ax + b$. Based on this curve, the concentration of ACE2 in each sample well can be derived with the following formulae:

1. Serum and Plasma samples:

One unit of ACE2 activity is defined as the amount of enzyme in 1 L of serum or plasma to produce 1 µmol of product per minute at 37°C.

$$\text{ACE2 activity (U/L)} = \mathrm{F} \times \frac{(\mathrm{F_{2\ Sample}} - \mathrm{F_{1\ Sample}} - \mathrm{b})}{\mathrm{a} \times \mathrm{t}}$$

2. Tissue Homogenates and Cell samples:

One unit of ACE2 activity is defined as the amount of enzyme in 1 g of sample protein to produce 1 µmol of product per minute at 37°C.

$$\text{ACE2 activity (U/g Protein)} = \mathrm{F} \times \frac{(\mathrm{F_{2\ Sample}} - \mathrm{F_{1\ Sample}} - \mathrm{b})}{\mathrm{a} \times \mathrm{t} \times \mathrm{C_{Protein}}}$$

where:

$F_{1\ Sample}$	Flourescence value of sample before incubation
$F_{1\ Standard}$	Flourescence value of standard before incubation
$F_{2\ Sample}$	Flourescence value of sample after incubation
$F_{2\ Standard}$	Flourescence value of standard after incubation
$C_{Protein}$	Concentration of protein in sample (g Protein/L)
a	Gradient of the standard curve ($y = \boldsymbol{a}x + b$)
b	Y-intercept of the standard curve ($y = ax + \boldsymbol{b}$)
t	Time of the enzymatic reaction (10 mins)
F	The dilution factor of sample

Technical Support

For troubleshooting and technical assistance, please contact us at support@abbexa.com.