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ACE1 Colorimetric Activity Assay Kit

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(Catalog # K2001-100; 100 assays; Store at -20 °C)

I. Introduction:

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. Previous researchers have shown that ACE activity is a better predictor of pathologies associated with Type 2 diabetes than ACE levels in serum samples. BioVision's 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 345 nm. The assay kit provides a rapid, simple and sensitive method to detect ACE1 activity as low as 40 mU in a variety of samples.

ACE1 Substrate Mix $\xrightarrow{\text{ACE1}}$ Product (**Decrease in OD: 345 nm**)

II. Application:

- Measurement of ACE1 activity in Plasma or Serum samples

III. Sample Types:

- Serum
- Plasma

IV. Kit Contents:

Components	K2001-100	Cap Code	Part Number
ACE1 Assay Buffer	25 ml	WM	K2001-100-1
ACE1 Substrate	1 vial	Brown	K2001-100-2
ACE1 Enzyme	50 µl	Green	K2001-100-3
96-Well Clear Bottom UV Plate	1	-	K2001-100-4

V. User Supplied Reagents and Equipment:

- Temperature-controlled microplate reader

VI. Storage Conditions and Reagent Preparation:

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

- **ACE1 Assay Buffer:** Warm to room temperature (RT) before use. Store at 4 °C.
- **ACE1 Substrate:** Reconstitute with 1.1 ml ACE1 assay buffer. Vortex to dissolve completely. Store at -20 °C.
- **ACE1 Enzyme:** Aliquot in 10 µl aliquots and store at -20 °C. Avoid multiple freeze/thaw of the enzyme. Use within 6 months.

VII. ACE1 Activity Assay Protocol:

1. ACE1 Enzyme Working Solution Preparation: Prepare a 20 fold dilution of the ACE1 Enzyme stock solution (2 µl of ACE1 Enzyme + 38 µl of ACE1 Assay Buffer). Mix thoroughly and keep on ice. Add Diluted ACE1 Enzyme Solution to each well(s) designated as Positive Control as specified in the Experimental design section. **Note:** Do not store the diluted ACE1 Enzyme Solution. Discard the unused solution.

2. Experimental Design: Add 30 µl of thawed Plasma or Serum Sample into well(s) designated as Test Sample in a 96-Well Clear Bottom UV Plate. For Blank, add 50 µl of ACE1 Assay Buffer. For Positive Control, add 40 µl of the diluted enzyme solution into desired well(s). Adjust the volume of Test Sample(s), Blank and Positive Control to **200 µl/well** with ACE1 Assay Buffer. Incubate at 37 °C for 10 min. **Note:** Plasma or Serum Samples can be used directly in the assay.

3. ACE1 Substrate Mix: Prepare a 5 fold dilution of the ACE1 Substrate with Assay Buffer (50 µl of ACE1 Substrate + 200 µl of ACE1 Assay Buffer). Dilute enough reagents for the number of assays to be performed. Mix and add 50 µl of diluted ACE1 Substrate mix into each Test Sample, Blank and Positive Control wells. Mix well and remove any bubbles. **Notes:** Diluted ACE1 Substrate can be stored at -20°C and used within 3 months.

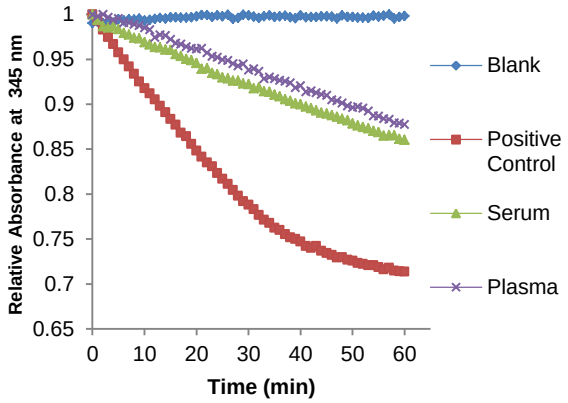
4. Measurement: Measure absorbance in kinetic mode at OD 345 nm for 60 min at 37°C. Samples having low ACE1 activity can be measured for 1.5 to 2 hr.

5. Calculation: Take the absorbance at 345 nm ($A_{1\ 345\ nm}$) and ($A_{2\ 345\ nm}$) at two time points (T_1 & T_2) respectively in the linear range. There should be at least two readings in between and at least 1 min apart. Note that the change in absorbance will be negative ($A_1 \geq A_2$). To determine the activity of ACE1, use the following equation:

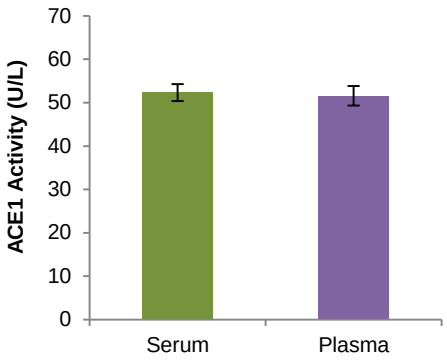
$$\text{Sample ACE1 Activity} = \frac{\left(\frac{|\Delta A_{345}|}{\Delta T}[Test\ Sample] - \frac{|\Delta A_{345}|}{\Delta T}[Blank]\right) \times (0.25) \times \text{DF}}{(0.34) \times \text{V}}\ \text{U/ml}$$

Where: $|\Delta A_{345}|$ = Absolute value of the change in absorbance ($A_{2\ 345\ nm} < A_{1\ 345\ nm}$)
ΔT = Difference between T_2 & T_1 (min)
0.25 = Reaction volume (ml)
DF = Sample Dilution factor (if applicable, D=1 for undiluted Samples)
0.34 = Millimolar extinction coefficient of ACE1 Substrate
V = Enzyme/Sample volume (ml)

A)



B)



Figures: A) Kinetic activity curves of ACE1 activity in Serum (30 µl) and Plasma (30 µl) Samples. *Note: Figure A curves were plotted after normalizing the raw values with the maximum absorbance value obtained from each sample.* B) ACE1 activity in human Serum and Plasma Samples (30 µl). Assays were performed following the kit protocol.

VIII. Related Products:

Angiotensin I Converting Enzyme Activity (ACE1) Assay Kit (K227)
Angiotensin I Converting Enzyme (ACE1) Inhibitor Screening Kit (K228)
Angiotensin II Converting Enzyme (ACE2) Inhibitor Screening Kit (K310)
ACE1 Inhibitor Screening Kit (Colorimetric) (K719)
Angiotensin II Converting Enzyme (ACE2) Activity Assay Kit (Fluorometric) (K897)
Angiotensin I (Human) ELISA Kit (E4515)
Angiotensin III (Ang III) (Human) ELISA Kit (E4536)
ACE2 (Human) ELISA Kit (E4528)
Angiotensin II (Ang II) (Human) ELISA Kit (E4527)

FOR RESEARCH USE ONLY! Not to be used on humans.