

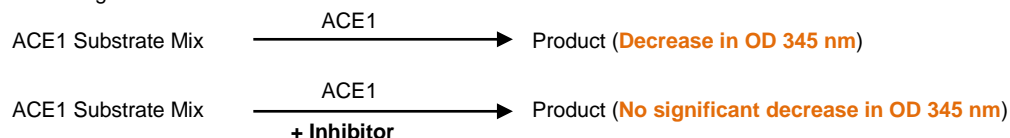
ACE1 Inhibitor Screening Kit (Colorimetric)

06/19

(Catalog # K719-100; 100 assays; Store at -20°C)

I. Introduction:

Angiotensin-I-converting enzyme (ACE) 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 that regulates blood pressure. ACE inhibitor inhibits ACE1 enzymatic activity and decreases the production of angiotensin II. As a result, blood vessels are dilated thereby increasing the amount of blood pumped by the heart and lowering the blood pressure. ACE inhibitors are used for treating hypertension, heart failure, stroke etc. Recent research is also focused on the discovery of natural products as ACE1 Inhibitors. BioVision's ACE1 Inhibitor Screening Kit can be used to screen potential ACE1 inhibitors. The kit utilizes the ability of an active ACE1 to hydrolyze a synthetic substrate, which results in the decrease in OD at 345 nm. In the presence of Captopril, an ACE1 specific inhibitor, the ACE1 enzymatic activity is greatly reduced and there is no significant decrease in the OD at 345 nm. The assay kit provides a rapid, simple and reliable test for high-throughput screening of ACE1 inhibitors.



II. Applications:

- Screening/characterizing ACE1 inhibitors

III. Kit Contents:

Components	K719-100	Cap Code	Part Number
ACE1 Assay Buffer	25 ml	WM	K719-100-1
ACE1 Substrate	1 vial	Amber	K719-100-2
ACE1 Enzyme	50 µl	Green	K719-100-3
ACE1 Inhibitor Control	100 µl	Blue	K719-100-4
96-Well UV Transparent Plate	1	-	K719-100-5

IV. User Supplied Reagents and Equipment:

- Temperature-controlled plate reader

V. Storage Conditions and Reagent Preparation:

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

- **ACE1 Assay Buffer:** Store at 4°C or -20°C. Bring to room temperature (RT) before use.
- **ACE1 Substrate:** Reconstitute with 1.1 ml ACE1 assay buffer. Vortex to dissolve completely. Store at -20°C. Thaw before use.
- **ACE1 Enzyme:** Aliquot in 10 µl aliquots and store at -20°C. Avoid multiple freeze-thaw cycles of the enzyme. Use within 6 months.
- **ACE1 Inhibitor Control (10 mM Captopril):** Aliquot and store at -20°C.
- **96-Well UV Transparent Plate:** Bring to RT before use.

VI. ACE1 Inhibitor Screening Protocol:

1. Test Inhibitor, Inhibitor Control Preparation: Dissolve Test Inhibitor(s) [S] at 100X in a proper solvent. For each Test Inhibitor, dilute to 10X the desired test concentration with ACE1 Assay Buffer. **To determine IC₅₀ values for Test Inhibitor(s), prepare several dilutions of the Test Inhibitor(s) in ACE1 Assay Buffer. Add 25 µl of each dilution into designated well(s).**

For Inhibitor Control, dilute the stock ACE1 Inhibitor Control (10 mM Captopril) to 100 µM Captopril by adding 5 µl of 10 mM Captopril to 495 µl Assay buffer. Prepare 1 µM working solution of Captopril by adding 5 µl of 100 µM of Captopril and 495 µl assay buffer. Add 25 µl of 1 µM Captopril into Inhibitor Control well(s). **Note:** Do not store the diluted Captopril solution. Discard the unused diluted solution.

Notes:

- Various organic solvents may affect the ACE1 enzymatic activity. We recommend preparing a parallel Solvent Control [SC] well used to at the final well concentration of the solvent used to test Inhibitor(s). If SC slope is significantly different from Enzyme Control [EC], use SC values to determine the effect of the respective Test Inhibitor (see Step 5).
- We recommend testing several dilutions of the diluted ACE1 Inhibitor Control [IC].

2. ACE1 Enzyme Solution Preparation: Prepare diluted ACE1 Enzyme Solution by adding 2 µl of the stock ACE1 Enzyme to 38 µl of ACE1 Assay Buffer. Mix thoroughly and keep on ice.

3. Screening Inhibitors: Prepare wells containing diluted Test Inhibitor(s) [S], Inhibitor Control [IC], Enzyme Control [EC], Background Control containing no enzyme [BC] and Solvent Control [SC].

	[S]	[IC]	[EC]	[BC]	[SC]
Test Inhibitor	25 µl	-	-	-	-
Inhibitor Control	-	25 µl	-	-	-
ACE1 Assay Buffer	-	-	25 µl	25 µl	-
Solvent Control	-	-	-	-	25 µl

Add 40 μ l of diluted ACE1 Enzyme Solution to each well containing [S], [IC], [EC] and [SC] only. Adjust the total volume of each well including [S], [IC], [EC], [SC] and [BC] to 200 μ l/well with ACE1 Assay Buffer. **Mix well and incubate at 37°C for 15-20 min, protected from light.** **Note:** Do not store diluted ACE1 Enzyme Solution. Discard the unused diluted Enzyme Solution.

4. Reaction Mix Preparation: Mix enough reagents for the number of assays to be performed. For each well, prepare 50 μ l Reaction Mix containing:

	Reaction Mix
ACE1 Assay Buffer	40 μ l
ACE1 Substrate Mix	10 μ l

Add 50 μ l Reaction Mix to [S], [IC], [EC], [SC] and [BC] wells and mix well.

5. Measurement: Measure the absorbance immediately at OD 345 nm in kinetic mode for 60 min at 37°C. Choose any two time points (t_1 & t_2) in the linear range of the plot and obtain the corresponding absorbance values (OD_1 & OD_2).

6. Calculation: Calculate the slope for [S], [EC], [SC] and [BC] by dividing the $\Delta OD = (OD_1 - OD_2)$ values by $\Delta t (t_2 - t_1)$. Subtract the Slope of BC] from [S], [EC] and [SC]. If [SC] slope is significantly different when compared to [EC], use [SC] values to determine effect of the Test Inhibitor.

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

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

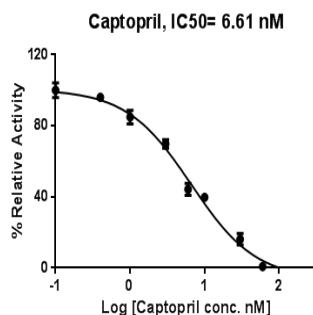


Figure: Inhibition of ACE1 activity by Captopril. IC₅₀ was calculated to be 6.61 nM. Assay was performed following the kit protocol.

VII. 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)
- Angiotensin II Converting Enzyme (ACE2) Activity Assay Kit (Fluorometric) (K897)
- ACE1 Colorimetric Activity Assay Kit (K2001)
- 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.