Angiotensin I Converting Enzyme (ACE1) Inhibitor Screening Kit

(Catalog # BN00501; 100 assays; Store at -20°C)

I. Introduction:

Angiotensin I converting enzyme (ACE1, EC: 3.4.15.1), a dipeptidyl carboxypeptidase, is part of the renin-angiotensin system (RAS) that controls regulation of blood pressure by cleaving the C-terminal dipeptides of angiotensin I and bradykinin. It is found on the luminal surface of vascular endothelial cells, especially in pulmonary tissues. In addition, elevated levels of ACE1 are found in sarcoidosis, leprosy, hyperthyroidism, acute hepatitis, primary biliary cirrhosis, diabetes mellitus, multiple myeloma, osteoarthritis, amyloidosis, Gaucher disease, pneumoconiosis, histoplasmosis and miliary tuberculosis. Assay Genie's ACE1 Inhibitor Screening Kit can be used to screen for potent inhibitors of ACE1 activity to regulate hypertension. It utilizes the ability of an active ACE1 to cleave a synthetic *o*-aminobenzoyl peptide (Abz-based peptide substrate to release a free fluorophore). The released Abz can be easily quantified using a fluorescence microplate reader. In the presence of an ACE1 specific inhibitor, the enzyme loses its peptidase activity which results in decrease of fluorescence intensity. This assay kit is simple and can be used to identify and characterize ACE1 inhibitors in a high-throughput format.

ACE1 Substrate-Abz $\xrightarrow{\text{ACE1}}$ Cleaved ACE1 Substrate + Abz (Ex/Em= 330/430 nm)

ACE1 Substrate-Abz $\xrightarrow[\text{+ ACE1 Inhibitor}]{\text{ACE1}}$ Decrease in Abz fluorescence/No fluorescence

II. Applications:

- Screening/characterizing inhibitors/ligands of ACE1

III. Kit Contents:

Components	BN00501	Cap Code	Part Number
ACE1 Assay Buffer	20 ml	WM	BN00501-1
ACE1 Dilution Buffer	1 ml	Clear	BN00501-2
ACE1 Enzyme	50 µl	Green	BN00501-3
ACE1 Substrate	300 µl	Brown	BN00501-4
ACE1 Inhibitor (10 mM Captopril)	100 µl	Blue	BN00501-5

IV. User Supplied Reagents and Equipment:

- 96-well white plate with flat bottom
- Multi-well Fluorescence microplate reader

V. Storage Conditions and Reagent Preparation:

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

- **ACE1 Assay Buffer and ACE1 Dilution Buffer:** Store at -20 °C or 4 °C. Bring to room temperature before use.
- **ACE1 Enzyme:** Store at -20°C. Avoid multiple freeze/thaw of the enzyme. Use within 6 months.
- **ACE1 Substrate:** Ready to use. Store at -20°C. Thaw before use.
- **ACE1 Inhibitor:** Ready to use. Store at -20°C. Thaw before use.

VI. ACE1 Inhibitor Screening Protocol:

1. **ACE1 Enzyme Working Solution Preparation**: Add 950 µl of ACE1 Dilution Buffer to the ACE1 enzyme vial. The diluted enzyme can be stored at -20°C in aliquots. For each well (Enzyme Control-EC, Sample-S, Inhibitor Control-IC), prepare 50 µl of ACE1 Enzyme Working Solution:

EC, S and IC	Background Control (BC)
40 µl ACE1 Assay Buffer	50 µl ACE1 Assay Buffer
10 µl Diluted ACE1 enzyme solution	-----

   Mix well and add 50 µl/well into desired wells in a 96-well microtiter plate.

2. **Screening Compounds, Inhibitor Control & Enzyme Control Preparations:** Dissolve candidate inhibitors at 100X highest final test concentration using preferred solvent. Dilute to 10X the desired test concentration with ACE1 Assay Buffer. Add 10 µl test inhibitors (Sample, S) or ACE1 Assay Buffer (Enzyme Control or BC). For Inhibitor Control (IC), add 10 µl ACE1 Inhibitor into ACE1 enzyme containing well(s). Incubate at 37ºC for 15 min.

   Note: High solvent concentration might affect the enzymatic activity. Prepare parallel well(s) as Solvent Control to test the effect of the solvent on enzyme activity. (same as EC in presence of final solvent concentration). In case SC is significantly different from EC, use its value in the calculations below.

3. **ACE1 Substrate Mix:** Prepare enough reagents for the number of assays to be performed. For each well, prepare 40 µl of the Substrate Mix:

   37 µl ACE1 Assay Buffer
   3 µl ACE1 Substrate

   Mix & add 40 µl of ACE1 Substrate Mix into Enzyme Control/Solvent Control, Inhibitor Control & Sample (S) wells. Mix well.

4. **Measurement:** Measure fluorescence (Ex/Em = 330/430 nm) in a kinetic mode for 1-2 hr at 37°C.

5. **Calculations**: Choose two time points (T_1 & T_2) in the linear range of the plot and obtain the corresponding values for the fluorescence (RFU_1 and RFU_2). Calculate the slope for all samples, ΔRFU/ΔT.

$$\%\ \textbf{Relative activity} = \frac{\textbf{Δ RFU of S}}{\textbf{ΔRFU of EC}} \times \textbf{100}$$

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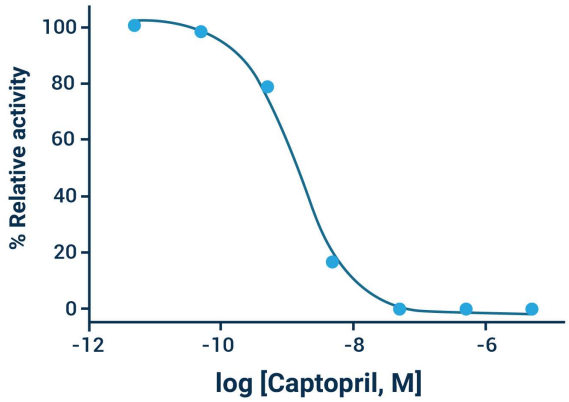

Figure: Inhibition of ACE1 activity by ACE1 Inhibitor Captopril (IC_{50} = 1.46 nM (n = 3)). Assay was performed following the kit protocol.

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