Angiotensin II Converting Enzyme (ACE2) Inhibitor Screening Kit (BN00569)

(Catalog BN00569; 100 assays; Store at -20°C)

I. Introduction:

Angiotensin II converting enzyme (ACE2, EC 3.4.17.23), a carboxypeptidase, is part of the renin-angiotensin system (RAS) that controls regulation of blood pressure by cleaving the C-terminal dipeptide of Angiotensin II to convert it into Angiotensin 1-7. It also cleaves Angiotensin I to produce Ang 1-9, of unknown function. ACE2 is a receptor of human coronaviruses, such as SARS and HCoV-NL63. It is expressed on the vascular endothelial cells of heart and kidney. The inhibitors of ACE2 could be able to regulate hypertension by changing vascular permeability. Screening for small molecule and peptide inhibitors might also help in finding treatment for coronavirus mediated infection. Assay Genie's ACE2 Inhibitor Screening Kit can be used to screen for potent inhibitors of ACE2 activity, it utilizes the ability of an active ACE2 to cleave a synthetic MCA based peptide substrate to release a free fluorophore. The released MCA can be easily quantified using a fluorescence microplate reader. In the presence of an ACE2 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 ACE2 inhibitors in a high-throughput format.

MCA-ACE2 Substrate $\xrightarrow{\text{ACE2}}$ Cleaved ACE2 Substrate + MCA (Ex/Em= 320/420 nm)

MCA-ACE2 Substrate $\xrightarrow[\text{+ ACE2 Inhibitor}]{\text{ACE2}}$ Decrease in MCA fluorescence/No fluorescence

II. Applications:

- Screening/characterizing inhibitors/ligands of ACE2

III. Kit Contents:

Components	BN00569	Cap Code
ACE2 Assay Buffer	25 ml	WM
ACE2 Dilution Buffer	1.5 ml	Clear
ACE2 Enzyme	20 µl	Green
ACE2 Substrate	200 µl	Brown
ACE2 Inhibitor (0.5 mM)	5 µl	Blue

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.

- **ACE2 Assay Buffer and ACE2 Dilution Buffer:** Store at -20 °C or 4 °C. Bring to room temperature before use.
- **ACE2 Enzyme:** Store at -20°C. Thaw before use. Avoid multiple freeze/thaw of the enzyme. Use within 3 months.
- **ACE2 Substrate:** Ready to use. Store at -20°C. Thaw before use.
- **ACE2 Inhibitor:** Store at -20°C. Thaw before use. Avoid multiple freeze/thaw of the inhibitor.

VI. ACE2 Inhibitor Screening Protocol:

1. **ACE2 Enzyme Working Solution Preparation**: Add 198 µl of ACE2 Dilution Buffer to the ACE2 enzyme vial. The diluted enzyme can be stored at -20°C in aliquots. For each well (Enzyme Control-EC, Sample-S, Inhibitor Control-IC and Solvent Control-SC), prepare 50 µl of ACE2 Enzyme Working Solution:

EC, S, SC and IC	Background Control (BC)
48 µl ACE2 Assay Buffer	50 µl ACE2 Assay Buffer
2 µl Diluted ACE2 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 ACE2 Assay Buffer. Add 10 µl test inhibitors (Sample, S) or ACE2 Assay Buffer (EC and BC). Prepare Inhibitor Control by adding 50 µl ACE2 Assay Buffer to the vial containing ACE2 Inhibitor, mix. For Inhibitor Control (IC), add 10 µl ACE2 Inhibitor into ACE2 enzyme containing well(s). Incubate at Room temperature (RT) 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. **ACE2 Substrate Mix:** Prepare enough reagents for the number of assays to be performed. For each well, prepare 40 µl of the Substrate Mix:

   38 µl ACE2 Assay Buffer
   2 µl ACE2 Substrate

   Mix & add 40 µl of ACE2 Substrate Mix into Background Control, Enzyme Control/Solvent Control, Inhibitor Control & Sample (S) wells. Mix well.

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4. **Measurement:** Measure fluorescence (Ex/Em = 320/420 nm) in a kinetic mode for 1 hr at room temperature.
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.

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

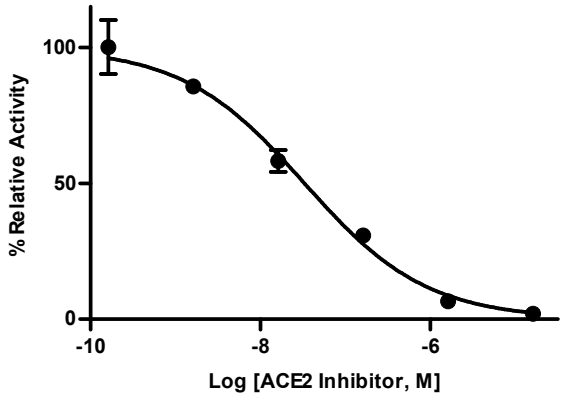

Figure: Inhibition of ACE2 activity by ACE2 Inhibitor, IC_{50} = 33.0 ± 0.6 nM (n = 3). Assay was performed following the kit protocol.

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