

## Description

The Fluoro-Verse™ Angiotensin Converting Enzyme 2 (ACE2) Inhibitor Assay Kit is a 96-well homogeneous fluorogenic assay designed to measure the exopeptidase activity of ACE2 for screening and profiling applications. The ACE2 assay kit contains enough purified ACE2 (BPS Bioscience #11003, amino acids 18-740), its substrate, and ACE2 buffer for 100 reactions.

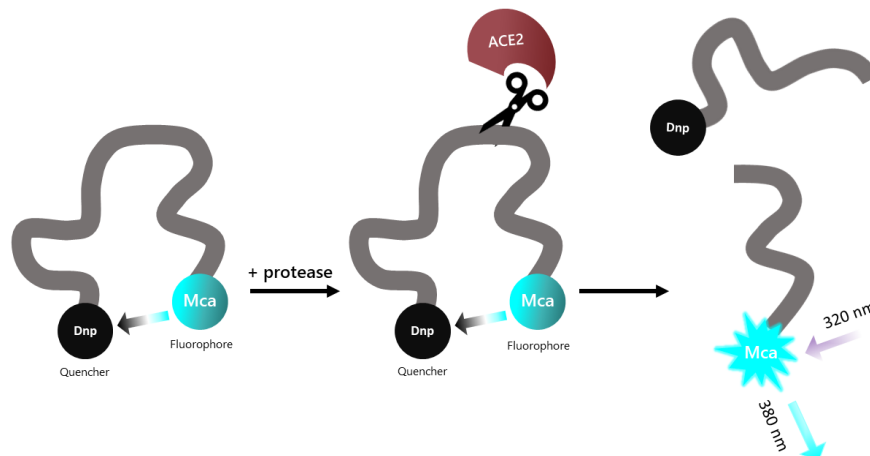


Figure 1: Illustration of the principle behind the ACE2 fluorogenic assay.

The assay uses an ACE2 substrate in which the fluorescence emitted by Mca (7-Methoxycoumarin-4-acetic acid) is quenched by the proximal Dnp (2,4-dinitrophenyl) quencher. ACE2 is a carboxypeptidase that cleaves the C-terminus peptide bond. Thus, Mca is released from quenching upon proteolysis and emits fluorescence with excitation/emission maxima of 320/380 nm. The increase in fluorescence is proportional to ACE2 activity.

## Background

Angiotensin Converting Enzyme 2 (ACE2) is an exopeptidase that catalyzes the conversion of angiotensin II to angiotensin 1-7 and L-phenylalanine. Angiotensin II is part of the classical renin angiotensin system (RAS), a hormone system that regulates fluid balance, blood pressure and maintains vascular tone. ACE2 plays a role in lowering blood pressure and in cardiovascular health and therefore, is an attractive therapeutic target. ACE2 is also the receptor for the human respiratory coronaviruses NL63, SARS (SARS-CoV) and 2019-nCoV/SARS-CoV-2.

## Applications

Screen small molecule inhibitors in high throughput screening (HTS) applications.

## Supplied Materials

Catalog #	Name	Amount	Storage
11003	ACE2, His-Tag*	>1 µg	-80°C
	ACE2 Fluorogenic Substrate	5 µl	-80°C
	ACE2 Buffer	3 ml	-20°C
79685	96-well black microplate	1	Room Temp

\* The concentration of protein is lot-specific and will be indicated on the tube containing the protein.

**Stability**

This assay kit will perform optimally for up to **6 months** from date of receipt when the materials are stored as directed.

**Safety**

This product is for research purposes only and not for human or therapeutic use. This product should be considered hazardous and is harmful by inhalation, in contact with skin, eyes, clothing, and if swallowed. If contact occurs, wash thoroughly.

**Assay Protocol**

- All samples and controls should be performed in duplicate.
- The assay should include a “Negative control”, a “Positive control” and a “Test inhibitor.”
- If the assay plate is going to be used more than once, prepare enough reagents for this portion of the assay and aliquot the remaining undiluted reagents into single-use aliquots depending on how many times the assay plate will be used. Store the aliquots at -80°C or at -20°C as appropriate.

1. Thaw **ACE2**, on ice. Briefly spin the tubes to recover the full content.
2. Dilute ACE2 to 0.05 ng/μl in ACE2 Buffer (20 μl/well). Keep the diluted protein on ice until use. Discard any unused diluted protein after use.
3. Prepare the Test Inhibitor (5 μl/well): for a titration, prepare serial dilutions at concentrations 10-fold higher than the desired final concentrations. The final volume of the reaction is 50 μl.

3.1 If the Test Inhibitor is water-soluble, prepare 10-fold more concentrated serial dilutions of the inhibitor than the desired final concentrations using the ACE2 Buffer. The ACE2 Buffer is the Diluent Solution.

**OR**

3.2 If the Test inhibitor is soluble in DMSO, prepare it in 100% DMSO at a concentration 100-fold higher than the highest desired concentration, then dilute 10-fold in ACE2 Buffer to prepare the highest concentration of the 10-fold intermediate dilutions. The concentration of DMSO is now 10%.

Prepare serial dilutions of the Test Inhibitor at concentrations 10-fold higher than the desired final concentrations using 10% DMSO in ACE2 Buffer to keep the concentration of DMSO constant.

For positive and negative controls, prepare 10% DMSO in ACE2 Buffer (vol/vol) so that all wells contain the same amount of DMSO (Diluent Solution).

*Note: The final concentration of DMSO should not exceed 1%.*

4. Add 20 μl of diluted ACE2 to the “Positive Control” and “Test Inhibitor.”
5. Add 20 μl of ACE2 Buffer to the “Negative Control.”

- Add 5  $\mu\text{l}$  of inhibitor solution to each well designated "Test Inhibitor."
- Add 5  $\mu\text{l}$  of Diluent Solution to the "Positive Control" and "Negative Control."
- Incubate the plate at room temperature for 30 minutes.
- Dilute **ACE2 Substrate** 500-fold in ACE2 buffer.
- Initiate the reaction by adding 25  $\mu\text{l}$  of the diluted ACE2 Substrate to all wells. Protect your samples from direct exposure to light and incubate at room temperature for 60 minutes.

Component	Negative control	Positive Control	Test Inhibitor
ACE2 Buffer	20 $\mu\text{l}$	-	-
Test inhibitor	-	-	5 $\mu\text{l}$
Diluent Solution	5 $\mu\text{l}$	5 $\mu\text{l}$	-
ACE2 (0.05 ng/ $\mu\text{l}$ )	-	20 $\mu\text{l}$	20 $\mu\text{l}$
Incubate at room temperature for 30 minutes			
ACE2 Substrate	25 $\mu\text{l}$	25 $\mu\text{l}$	25 $\mu\text{l}$
Total	50 $\mu\text{l}$	50 $\mu\text{l}$	50 $\mu\text{l}$

- Read fluorescence intensity of the samples (lexcitation = 320 nm; lemission = 380 nm) in a fluorescence plate reader.

## Example Results

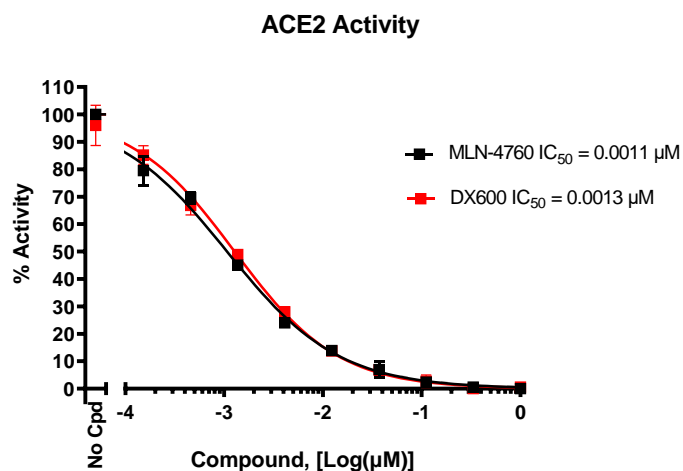


Figure 2. Inhibition of ACE2 activity.

ACE2 activity was measured in the presence of increasing concentrations of DX600 (Cayman #22186) and MLN-4760 (Sigma #530616). Fluorescence was measured using a Bio-Tek microplate reader. Results are expressed as percent on control (no inhibitor, set at 100%).

Data shown is representative. For lot-specific information, please contact BPS Bioscience, Inc. at [support@bpsbioscience.com](mailto:support@bpsbioscience.com)

**Troubleshooting Guide**

Visit [bpsbioscience.com/assay-kits-faq](https://bpsbioscience.com/assay-kits-faq) for detailed troubleshooting instructions. For further questions, please email [support@bpsbioscience.com](mailto:support@bpsbioscience.com)

**References**

Jackson C., *et al.* 2022, Mechanisms of SARS-CoV-2 entry into the cells, *Nature* 23: 3-20

**Related Products**

<i>Products</i>	<i>Catalog #</i>	<i>Size</i>
3CL Protease (B.1.1.529, Omicron Variant) (SARS-CoV-2)	101328	100 µg/1 mg
3CL Protease, Untagged (SARS-CoV-2) Assay Kit	78042	96 reactions/384 reactions
3CL Protease, MBP-tagged (SARS-CoV-2) Assay Kit	79955	96 reactions/384 reactions
ACE2, His-tag	11003	10 µg
PRCP, His-tag	80380	10 µg