

Description

The ACE2 Inhibitor Screening Assay Kit is a 96-well homogeneous fluorogenic assay designed to measure the exopeptidase activity of ACE2 (Angiotensin converting enzyme 2) for screening and profiling applications. The ACE2 kit contains enough purified ACE2 (amino acids 18-740), ACE2 substrate, and ACE2 buffer for 100 reactions.

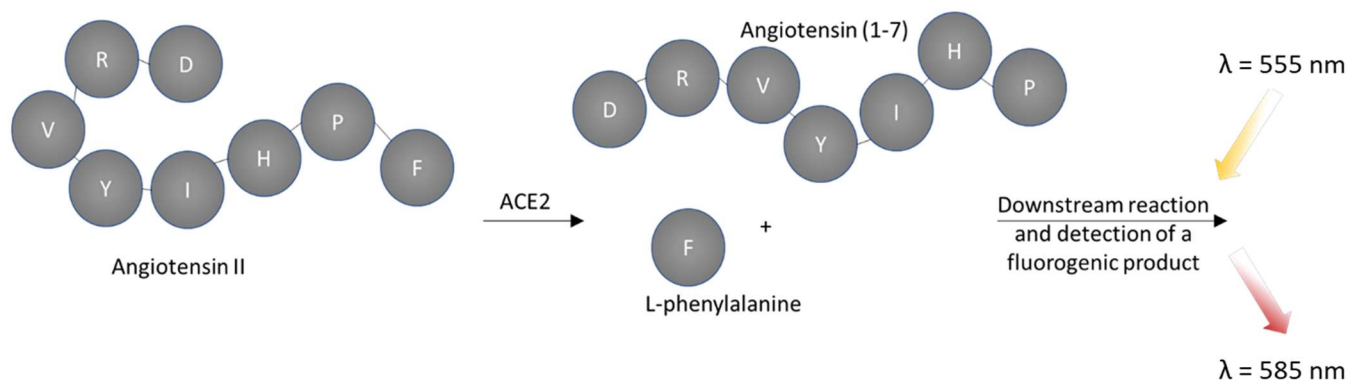


Figure 1: Illustration of the assay principle.

Cleavage of the peptide by ACE2 releases L-phenylalanine, which is measured by a two-step reaction and detection of a fluorogenic product. The fluorescence signal increases in proportion to ACE2 activity.

Note: For an assay with higher sensitivity, please refer to Fluoro-Verse™ ACE2 Inhibitor Assay Kit (#78847).

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

Enzyme kinetics and screening of small molecule inhibitors in high throughput screening (HTS) applications.

Supplied Materials

Catalog #	Name	Amount	Storage
11003	ACE2, His-Tag*	2 µg	-80°C
	ACE2 Fluorogenic Substrate	2.5 ml	-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.

Contraindications

The final concentration of DMSO in the assay should not exceed 1%.

Assay Protocol

- All samples and controls should be performed in duplicate.
- The assay should include “Negative Control”, “Positive Control” and “Test Inhibitor” conditions.
- We recommend maintaining the diluted protein on ice during use.
- For detailed information on protein handling please refer to [Protein FAQs \(bpsbioscience.com\)](https://bpsbioscience.com).
- We recommend using DX600 as internal control. If not running a dose response curve for the control inhibitor, we recommend running the control inhibitor at 0.1X, 1X and 10X the IC₅₀ value shown in the validation data below.

1. Thaw **ACE2** on ice. Briefly spin the tube to recover the full content.
2. Dilute ACE2 to 0.5 ng/μl with ACE2 Buffer (20 μl/well).
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 serial dilutions of the inhibitor 10-fold more concentrated than the desired final concentrations using the ACE2 Buffer.

For the positive and negative controls, use ACE2 Buffer (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 it 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, add 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 μ l of diluted ACE2 to the “Positive Control” and “Test Inhibitor” wells.
5. Add 20 μ l of ACE2 Buffer to the “Negative Control” wells.
6. Add 5 μ l of Test inhibitor dilutions to each well designated “Test Inhibitor”.
7. Add 5 μ l of Diluent Solution to the “Positive Control” and “Negative Control” wells.
8. Thaw **ACE2 Fluorogenic Substrate** on ice. Briefly spin the tube to recover the full content.
9. Initiate the reaction by adding 25 μ l of **ACE2 Fluorogenic Substrate** to all wells.
10. Incubate at room temperature for 60 minutes.



Protect your samples from direct exposure to light.

Component	Negative control	Positive Control	Test Inhibitor
ACE2 Buffer	20 μ l	-	-
Test inhibitor	-	-	5 μ l
Diluent Solution	5 μ l	5 μ l	-
Diluted ACE2 (0.5 ng/ μ l)	-	20 μ l	20 μ l
ACE2 Substrate	25 μ l	25 μ l	25 μ l
Total	50 μl	50 μl	50 μl

11. Read fluorescence intensity of the samples ($\lambda_{excitation}$ = 555 nm; $\lambda_{emission}$ = 585 nm) in a fluorescence plate reader.

Example Results

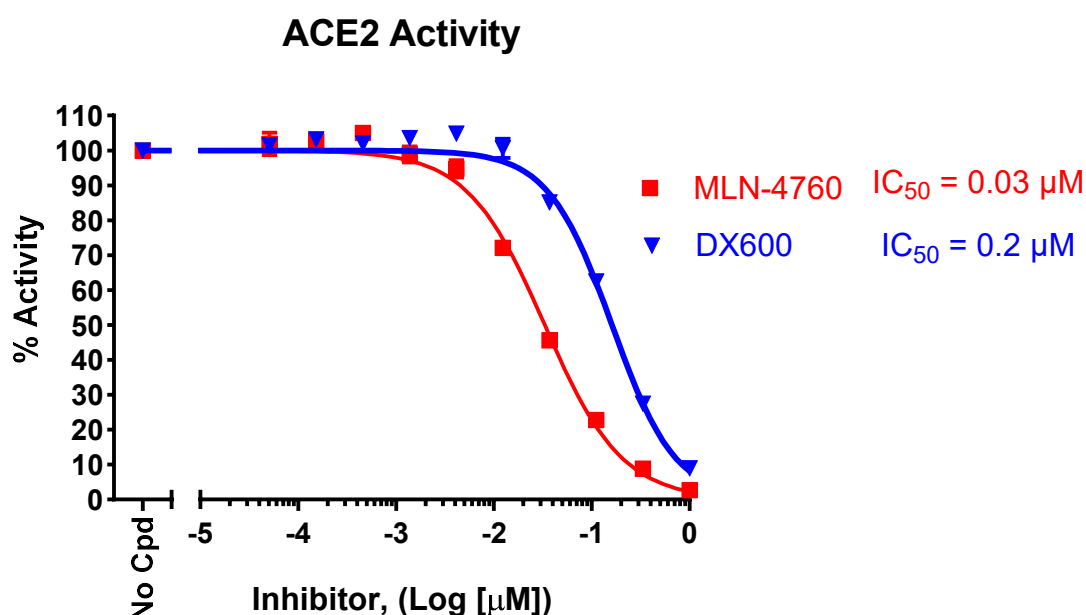


Figure 2. Inhibition of ACE2 activity by MLN-4660 and DX600.

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

Troubleshooting Guide

Visit bpsbioscience.com/assay-kits-faq for detailed troubleshooting instructions. For further questions, please email support@bpsbioscience.com

References

Jackson C., *et al.*, 2022 *Nature* 23: 3-20

Related Products

Products	Catalog #	Size
Fluoro-Verse™ ACE2 Inhibitor Assay Kit	78847	96 reactions
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 Recombinant	11003	10 μg
PrCP, His-Tag Recombinant	80380	10 μg

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