Description

The ACE2 Inhibitor Screening Assay Kit is a fluorogenic assay designed to measure the exopeptidase activity of ACE2 for screening and profiling applications. The ACE2 assay kit contains sufficient amounts of purified ACE2, fluorogenic ACE2 peptide substrate, and ACE2 assay buffer for 96 reactions.

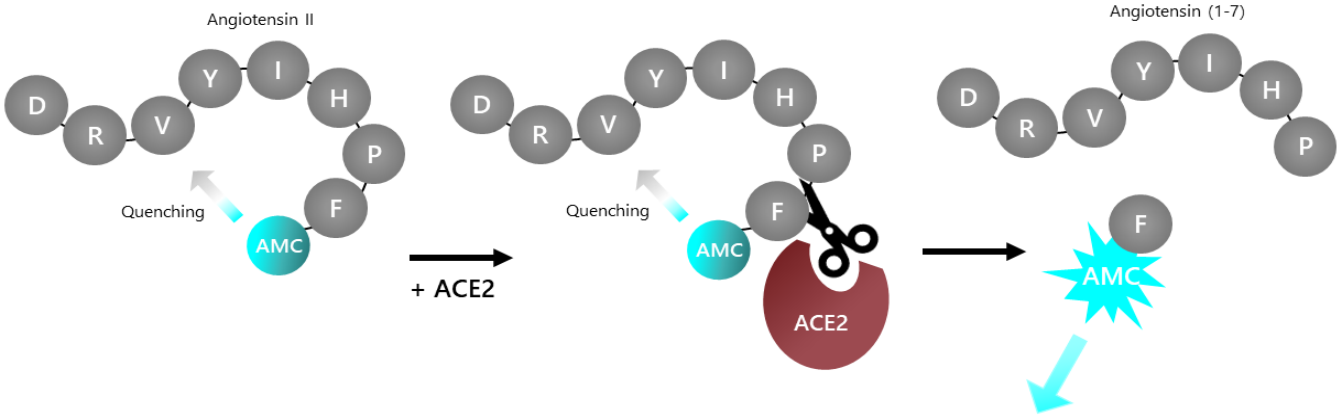

Illustration of the assay principle.

The substrate is an internally quenched fluorogenic substrate. When fluorophore AMC is attached to the short peptide, its fluorescence is quenched. AMC is freed upon proteolysis and highly fluorescent. Fluorescence intensity increases proportionally to the activity of the protease (Excλ=550nm; Emλ=585nm).

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, a hormone system that regulates fluid balance, blood pressure and maintains vascular tone. ACE2 is also the host receptor for the human respiratory coronavirus NL63, the SARS-coronavirus (SARS-CoV) and the novel coronavirus 2019-nCoV/SARS-CoV-2. Blocking the binding of viral proteins to ACE2 is a validated therapeutic approach against these respiratory infectious diseases.

Applications

Screen small molecular inhibitors of ACE2 peptidase activity in high throughput applications.

Supplied Materials

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

Materials Required but Not Supplied

Name	Ordering Information
Microplate reader capable of reading fluorescent intensity Adjustable micropipettor and sterile tips	

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 duplicates.
- The assay should include a "Negative control", a "Positive control", and a "Test inhibitor."

1. Thaw ACE2 enzyme on ice. Briefly spin the tube to recover its full contents. If the assay plate is going to be used more than once, prepare enough enzyme for this portion of the assay and aliquot the remaining undiluted enzyme into single-use aliquots depending on how many times the assay plate will be used. Store the aliquots at -80°C.

2. Dilute ACE2 to 0.5 ng/µl in ACE2 Assay Buffer (you need 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.
   a) 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 Assay Buffer. For the positive and negative controls, use ACE2 Assay Buffer (Diluent Solution).
   OR
   b) If the Test inhibitor is soluble in DMSO, prepare the test inhibitor in 100% DMSO at a concentration 100-fold higher than the highest desired concentration, then dilute the inhibitor 10-fold in ACE2 Assay Buffer to prepare the highest concentration of the 10-fold intermediate dilutions. The concentration of DMSO is now 10%.
   For positive and negative controls, prepare 10% DMSO in Assay Buffer (vol/vol) so that all wells contain the same amount of DMSO (Diluent Solution).
   The final concentration of DMSO should not exceed 1%.

4. Add 20 µl of diluted ACE2 to all wells except "Negative control."

5. Add 20 µl of Assay Buffer to "Negative control."

6. Add 5 µl of Test Inhibitor solution to each well designated "Test Inhibitor."

7. For the "Positive Control," and "Negative Control" add 5 µl of Diluent Solution (for example, 10% DMSO in ACE2 Assay Buffer if the inhibitor was dissolved in DMSO).

8. Initiate the reaction by adding 25 µl of ACE2 Fluorogenic Substrate to all the wells. and incubate at room temperature for 60 minutes.

Protect the fluorogenic peptide and the reaction from direct exposure to light.

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

9. Read the fluorescence intensity of the samples (lexcitation=555 nm; lemission=585 nm) in a fluorescent plate reader.

Example Results

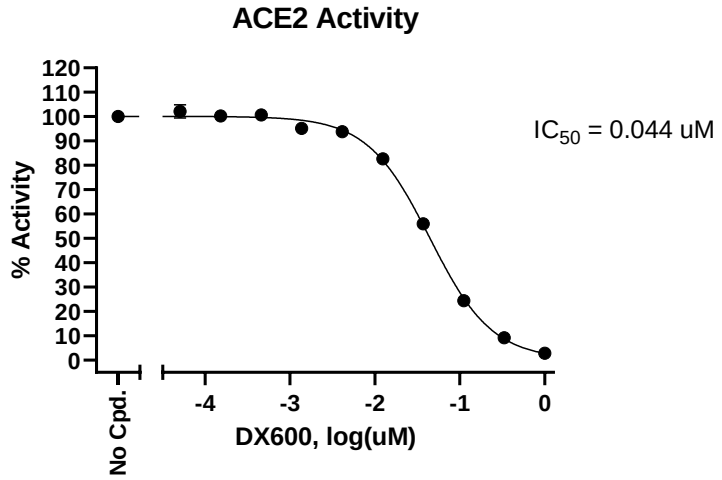

Figure 1. Inhibition of ACE2 peptidase activity by DX600.
ACE2 enzymatic activity was measured in the presence of increasing concentrations of DX600 (Cayman #22186).

Data shown is representative. For lot-specific information, please contact info@amsbio.com

References

1. Danilczyk U., *et al*. Circulation Research. 2006. 98(4): 463-471.
2. Ge XY., *et al.* Nature. 2013. 503(7477): 535-538.

Products	*Catalog #*	*Size*
ACE2, His-Tag	11003	20 µg/100 µg
PLPro, His-Tag (SARS-CoV-2) Recombinant	100735	100 µg/1 mg
3CL Protease (SARS-CoV-2) Recombinant	100823	50 µg/ 500 µg
3CL Protease, Untagged (SARS-CoV-2) Assay Kit	78042	96 reactions/384 reactions
3CL Protease (B.1.1.529, Omicron Variant) (SARS-CoV-2) Assay Kit	78350	96 reactions/384 reactions

AMSBIO | www.amsbio.com | info@amsbio.com

AMSBIO LLC
USA & Canada
1035 Cambridge Street,
Cambridge, MA 02141
T: +1 (617) 945-5033 or
T: +1 (800) 987-0985
F: +1 (617) 945-8218

AMSBIO Europe BV
EU
Berenkoog 41,
1822 BH Alkmaar,
Netherlands
T: +31 (0) 72 8080244
F: +31 (0) 72 8080142

AMS Biotechnology (Europe) Ltd
UK & Rest of the World
184 Park Drive, Milton Park
Abingdon OX14 4SE
T: +44 (0) 1235 828 200
F: +44 (0) 1235 820 482

AMS Biotechnology (Europe) Ltd
Switzerland
Via Lisano 3,
(CP.683)
CH-6900 Massagno
T: +41 (0) 91 604 55 22
F: +41 (0) 91 605 17 85

AMSBIO Europe BV
Deutschland
T: +49 (0) 69 779099
F: +49 (0) 69 13376880