Data Sheet
ACE2 Inhibitor Screening Assay Kit
Catalog #AMS.79923
Size: 96 reactions

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 has been also proved to be the receptor for the human respiratory coronavirus NL63, the SARS-coronavirus (SARS-CoV) and the novel coronavirus 2019-nCoV/SARS-CoV-2.

DESCRIPTION: The *ACE2 Inhibitor Screening Assay Kit* is designed to measure the exopeptidase activity of ACE2 for screening and profiling applications. The ACE2 assay kit comes in a convenient 96-well format, with purified ACE2, its substrate, and ACE2 buffer for 96 reactions.

COMPONENTS:

Catalog #	Component	Amount	Storage	
11003	ACE2	2 µg	-80 °C	Avoid multiple freeze/thaw cycles!
	ACE2 Fluorogenic Substrate	3 ml	-80 °C	
	ACE2 Buffer	3 ml	-20 °C	
79685	96-well black microplate	1	Room Temp	

MATERIALS OR INSTRUMENTS REQUIRED BUT NOT SUPPLIED:
Adjustable micropipettor and sterile tips
Fluorescent microplate reader

APPLICATIONS: Useful for studying enzyme kinetics and screening small molecule inhibitors and antibodies for drug discovery and HTS applications.

STABILITY: Up to 6 months from date of receipt, when stored as recommended.

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

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ASSAY PROTOCOL:
All samples and controls should be tested in duplicate.

1. Thaw **ACE2** on ice. Upon first thaw, briefly spin tube containing enzyme to recover full contents of the tube. Note: **ACE2** is sensitive to freeze/thaw cycles. Avoid multiple freeze/thaw cycles. Do not re-use thawed aliquots or diluted enzyme.
2. Prepare **Enzyme solution** (0.5 ng/µl ACE2) by diluting ACE2 in **ACE2 Buffer**.
3. Add 20 µl of **Enzyme solution** (0.5 ng/µl ACE2) to each well designated "Positive Control" and "Test Inhibitor," and 20 µl of **ACE2 buffer** to each well designated "Blank."
4. Add 5 µl of **Test Inhibitor** solution to each well designated "Test Inhibitor." For the wells labeled "Positive Control" and "Blank," add 5 µl of 10% DMSO in water (Inhibitor buffer). Note: Keep the final concentration of DMSO in the reaction ≤1%.
5. Add 25 µl of **ACE2 Fluorogenic Substrate** to all wells. Protect from light and incubate reaction at room temperature for 60 minutes.

	Positive Control	**Test Inhibitor**	**Blank**
Enzyme solution (0.5 ng/µl ACE2)	20 µl	20 µl	-
ACE2 Buffer	-	-	20 µl
Test inhibitor	-	5 µl	-
10% DMSO in water (Inhibitor buffer)	5 µl	-	5 µl
ACE2 Fluorogenic Substrate	25 µl	25 µl	25 µl
Total	**50 µl**	**50 µl**	**50 µl**

6. Read fluorescence intensity of the samples ($\lambda_{excitation}$ = 555 nm; $\lambda_{emission}$ = 585 nm) in an appropriate microplate reader.

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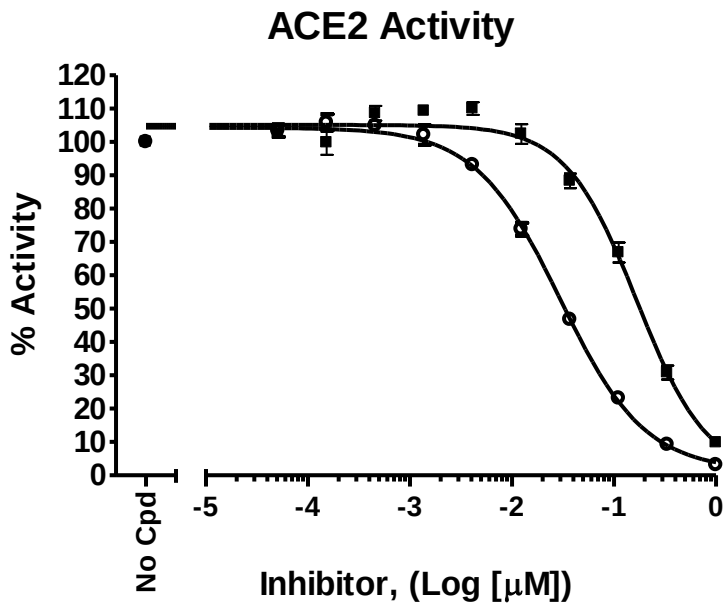
Example of assay results:



- DX600, IC50 = 0.17 µM
- MLN-4760, IC50 = 0.03 µM

ACE2 inhibition by DX600 (Cayman, #22186) and MLN-4760 (Sigma, #5306160001) measured using the *ACE2 Inhibitor Screening Assay Kit*, AMSBIO #AMS.79923. Fluorescencewas measured using a Bio-Tek microplate reader. Data shown is lot-specific. For lot-specific information, please contact AMSBIO at info@amsbio.com.