

Data sheet
SARS-CoV-2 Spike S1-Biotin:
ACE2 TR-FRET Assay Kit
Catalog # AMS.79949-1
Size: 96 reactions

DESCRIPTION: The pandemic coronavirus disease 2019 (COVID-19) is caused by Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2). As a first step of the viral replication strategy, the virus attaches to the host cell surface before entering the cell. The Spike protein recognizes and attaches to the Angiotensin-Converting Enzyme 2 (ACE2) receptor found on the surface of type I and II pneumocytes, endothelial cells, and ciliated bronchial epithelial cells. Drugs targeting the interaction between the Spike protein of SARS-CoV-2 and ACE2 may offer some protection against the viral infection.

The SARS-CoV-2 Spike S1:ACE2 TR-FRET Assay is designed to measure the inhibition of the binding between SARS-CoV-2 Spike S1 and human ACE2 in a homogeneous 96 reaction format. This TR-FRET-based assay requires no time-consuming washing steps, making it especially suitable for high throughput screening applications. The assay procedure is straightforward and simple; the test inhibitor compound is incubated with biotinylated Spike S1, Eu-labeled ACE2, dye-labeled acceptor and an inhibitor for one hour. Then the TR-FRET signal is measured using a fluorescence reader.

COMPONENTS:

Catalog #	Component	Amount	Storage	
100705	ACE2, His-Tag, Eu-labeled	2 µg	-80°C	Avoid multiple freeze/thaw cycles!
100679	Spike S1, Fc fusion, Avi-tag, Biotin-Labeled (SARS-CoV-2)	25 µg	-80°C	
	Dye-labeled acceptor	2 x 10 µl	-20°C	
79953	3x ACE2-Spike TR-FRET Buffer	4 ml	-20°C	
	96-well white microplate	1	Room Temp	

APPLICATIONS: This kit is useful for screening for inhibitors of the interaction between SARS-CoV-2 Spike S1 and human ACE2.

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

REFERENCES:

Hoffmann, M. *et al.* 2020. *Cell*, **181**:1-10
 Yan, R. *et al.* 2020. *Science* **367(6485)**:1444-1448.

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MATERIALS OR INSTRUMENTS REQUIRED BUT NOT SUPPLIED:

Fluorescence microplate reader capable of measuring Time Resolved-Fluorescence Resonance Energy Transfer (TR-FRET)

Adjustable micropipettor and sterile tips

ASSAY PROTOCOL:

All samples and controls should be tested in duplicate.

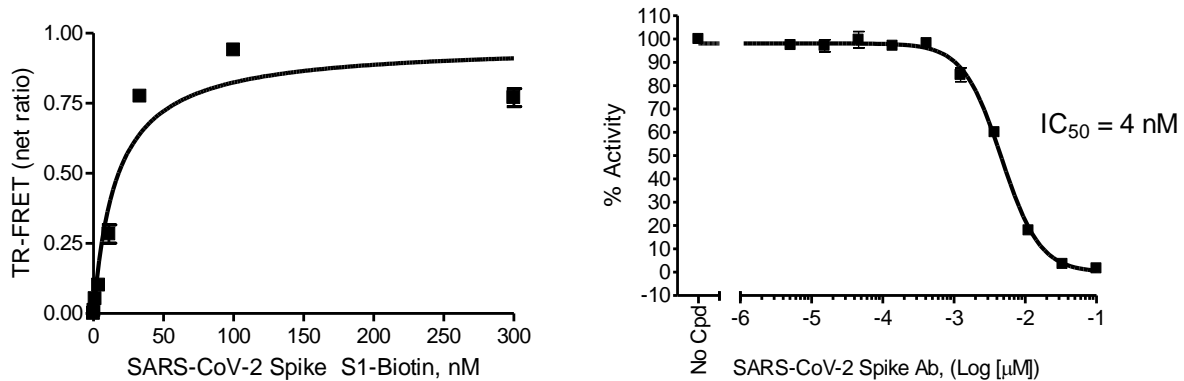
- 1) Thaw **ACE2-Eu** on ice. Upon first thaw, briefly spin tube containing the protein to recover the full contents of the tube. Aliquot into single use aliquots. Immediately store remaining undiluted protein in aliquots at -80°C . Note: **ACE2-Eu** is very sensitive to freeze/thaw cycles. Avoid multiple freeze/thaw cycles.
- 2) Dilute one part **3x ACE2-Spike TR-FRET Buffer** with 2 parts of distilled water (3-fold dilution) to make **1x ACE2-Spike TR-FRET Buffer**. Prepare only a sufficient quantity needed for the assay; store remaining stock solution in aliquots at -20°C .
- 3) Dilute **ACE2-Eu** in **1x ACE2-Spike TR-FRET Buffer** to $1\text{ ng}/\mu\text{l}$ (12 nM). Keep diluted protein on ice until ready to use. Discard any remaining unused diluted protein after use. Add $12.5\ \mu\text{l}$ of **ACE2-Eu** to all wells
- 4) Dilute **Dye-labeled Acceptor** 100-fold with **1x ACE2-Spike TR-FRET Buffer**. Add $12.5\ \mu\text{l}$ of **Dye-labeled Acceptor** to all wells
- 5) Prepare the **Test inhibitor** solution. If the inhibitor compound is water soluble (e.g. an antibody), make a solution of the compound 4-fold higher than the final concentration in **1x ACE2-Spike TR-FRET Buffer**. If the inhibitor compound is a small molecule soluble in DMSO, final DMSO concentration in the assay should be $\leq 1\%$, and Inhibitor buffer should contain the same concentration of DMSO as the test inhibitor.
- 6) Add $12.5\ \mu\text{l}$ of test inhibitor solution to each well designated "Test Inhibitor". For the "Positive Control" and "Blank", add $12.5\ \mu\text{l}$ of the same solution without inhibitor (**1x ACE2-Spike TR-FRET Buffer** with the same concentration of solvent as in the test inhibitor solution).

	Positive Control	Blank	Test Inhibitor
ACE2-Eu (1 ng/μl)	12.5 μl	12.5 μl	12.5 μl
Dye-labeled acceptor	12.5 μl	12.5 μl	12.5 μl
Test Inhibitor	-	-	12.5 μl
Inhibitor buffer	12.5 μl	12.5 μl	-
1x TR-FRET Buffer	-	12.5 μl	-
Spike S1-Biotin (20 ng/μl)	12.5 μl	-	12.5 μl
Total	50 μl	50 μl	50 μl

- 7) Thaw **Spike S1-Biotin** on ice. Upon first thaw, briefly spin tube containing the protein to recover the full contents of the tube. Aliquot into single use aliquots. Immediately store remaining undiluted protein in aliquots at -80°C. Note: **Spike S1-Biotin** is very sensitive to freeze/thaw cycles. Avoid multiple freeze/thaw cycles.
- 8) Dilute **Spike S1-Biotin** in **1x ACE2-Spike TR-FRET Buffer** to 20 ng/μl (200 nM). Keep diluted protein on ice until ready to use. Discard any remaining unused diluted protein after use. Add 12.5 μl of diluted **Spike S1-Biotin** to wells designated “Test Inhibitor” and “Positive Control”. Add 12.5 μl of **1x ACE2-Spike TR-FRET Buffer** to wells designated “Blank”. Incubate the plate at room temperature for 1 hour.
- 9) Read TR-FRET signal in a microtiter-plate reader under settings described below (settings may need optimization depending on the instrument). Blank value is subtracted from all other values.

Channel	Variable	Recommended value
1	Excitation wavelength (nm)	340 ± 20
	Emission wavelength (nm)	620 ± 10
	Lag time (μs)	60
	Integration time (μs)	500
2	Excitation wavelength (nm)	340 ± 20
	Emission wavelength (nm)	665 ± 10
	Lag time (μs)	60
	Integration time (μs)	500

Example of assay results:



Titration of SARS-CoV-2 Spike S1 (AMSBIO, #100679) (left) and inhibition of SARS-CoV-2 Spike S1:ACE2 binding using human anti-SARS-CoV-2 Spike Antibody (AMSBIO,#100793) (right) in the *SARS-CoV-2 Spike S1:ACE2 TR-FRET Assay Kit* (AMSBIO,#79949-1). Data shown is lot-specific.

RELATED PRODUCTS:

Product Name	Catalog#	Size
Spike S1-Biotin (SARS-CoV-2): ACE2 TR-FRET Assay Kit	79949-2	384 reactions
SARS-CoV-2 Spike:ACE2 Inhibitor Screening Assay Kit	79931	96 reactions
ACE2:SARS-CoV-2 Spike Inhibitor Screening Assay Kit	79936	96 reactions
ACE2:SARS-CoV-2 Spike S1-Biotin Inhibitor Screening Assay Kit	79945	96 reactions
Spike S1, Fc Fusion, Avi-tag (SARS-CoV-2)	100678	100 µg/1 mg
Spike S1, Fc fusion, Avi-tag, Biotin-Labeled (SARS-CoV-2)	100679	25 µg/50 µg
Spike S1 RBD, His-tag (SARS-CoV-2)	100687	50 µg/100 µg
Spike S1, Fc fusion (SARS-CoV-2)	100688	20 µg/50 µg
Spike S1 RBD, Fc fusion (SARS-CoV-2)	100699	50 µg/100 µg
ACE2 Inhibitor Screening Assay Kit	79923	96 reactions
ACE2, His-tag	11003	20 µg/100 µg
ACE2, His-Avi-Tag, Biotin-labeled HiP™	100665	20 µg/50 µg
ACE2, Fc Fusion (Monkey)	100701	50 µg/1 mg
ACE2, His-tag (Monkey)	100702	50 µg/1 mg

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