6405 Mira Mesa Blvd. Suite 100
San Diego, CA 92121
Tel: 1.858.202.1401
Fax: 1.858.481.8694
Email: support@bpsbioscience.com

Data sheet
ACE2:SARS-CoV-2 Spike S1 Inhibitor Screening Assay Kit
Catalog #79945
Size: 96 reactions

DESCRIPTION: Coronavirus disease 2019 (COVID-19) increases the risk of developing Acute Respiratory Distress Syndrome (ARDS), which is often fatal at the late stages of the infection when the SAR-CoV-2 virus causes significant damage to the lungs. As a first step of the viral replication strategy, the virus attaches to the host cell surface before entering the cell. The Spike S1 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 S1 protein of SARS-CoV-2 and ACE2 may offer some protection against the viral infection.

The ACE2:SARS-CoV-2 Spike S1 Inhibitor Screening Assay Kit is designed for screening and profiling inhibitors of this interaction. This kit comes in a convenient 96-well format, with purified ACE2 and SARS-CoV-2 Spike S1 proteins, Streptavidin-HRP, and assay buffer for 100 binding reactions. The key to this kit is the high sensitivity of detection of Spike S1-Biotin protein by Streptavidin-HRP. Only a few simple steps on a microtiter plate are required for the assay. First, ACE2 protein is attached to a nickel-coated 96-well plate. Next, SARS-CoV-2 Spike S1-Biotin is incubated with ACE2 on the plate. Finally, the plate is treated with Streptavidin-HRP followed by addition of an HRP substrate to produce chemiluminescence, which then can be measured using a chemiluminescence reader.

COMPONENTS:

Catalog #	Component	Amount	Storage	
100679	SARS-CoV-2 Spike S1, Fc Fusion, Avi-tag, Biotin-labeled*	10 µg	-80°C	Avoid multiple freeze/ thaw cycles!
11003	ACE2, His-Tag*	5 µg	-80°C	
79311	3x Immuno Buffer 1	50 ml	-20°C	
79728	Blocking Buffer 2	50 ml	+4°C	
79742	Streptavidin-HRP	10 µl	+4°C	
79670	ELISA ECL substrate A (transparent bottle)	6 ml	Room temp	
	ELISA ECL substrate B (brown bottle)	6 ml	Room temp	
	Nickel-coated 96-well white microplate	1	+4°C	

**The concentrations of Spike S1 and ACE2 are lot-specific and will be indicated on the tubes containing the protein.*

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APPLICATIONS: This kit is useful for screening for inhibitors of ACE2 binding to SARS-CoV-2 Spike S1.

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.

MATERIALS OR INSTRUMENTS REQUIRED BUT NOT SUPPLIED:
PBS (Phosphate buffered saline)
Luminometer or microplate reader capable of reading chemiluminescence
Adjustable micropipettor and sterile tips

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

Coating the plate with ACE2-His:

1) Thaw **ACE2-His** on ice. Upon first thaw, briefly spin tube containing **ACE2-His** to recover the full contents of the tube. Aliquot into single use aliquots. Immediately store remaining **ACE2-His** in aliquots at -80°C. Note: **ACE2-His** is very sensitive to freeze/thaw cycles. Avoid multiple freeze/thaw cycles.
2) Dilute **ACE2-His** to 1 µg/ml in PBS.
3) Add 50 µl of diluted **ACE2-His** solution to each well and incubate at room temperature for one hour with slow shaking.
4) Dilute **3x Immuno Buffer 1** to **1x Immuno Buffer 1** with water. Dilute only the amount required for the assay; store remaining **3x Immuno Buffer 1** undiluted.
5) Decant to remove supernatant. Wash the plate three times with 100 µl **1x Immuno Buffer 1**. Tap plate onto clean paper towels to remove liquid.

Block wells by adding 100 µl of **Blocking Buffer 2** to each well. Incubate for 10 minutes at room temperature with slow shaking. Remove supernatant as described in step 5.

Step 1:

1) Add 20 µl of **1x Immuno Buffer 1** to each well.
2) Add 10 µl of inhibitor solution to each well designated “Test Inhibitor”. For the “Positive Control” and “Blank”, add 10 µl of the same solution without inhibitor (inhibitor buffer). Optionally, incubate at room temperature for one hour with slow shaking.

   Note: It is recommendable to use PBS to dilute antibodies or other proteins acting as neutralization inhibitors. When using small molecules dissolved in DMSO, final DMSO

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concentration in the assay should be ≤1%. Inhibitor buffer should contain the same concentration of DMSO as the test inhibitor.

3) Thaw **SARS-CoV-2 Spike S1-Biotin** on ice. Upon first thaw, briefly spin tube containing enzyme to recover full contents of the tube. Aliquot **SARS-CoV-2 Spike S1-Biotin** into single use aliquots. Immediately store remaining undiluted enzyme in aliquots at -80°C. Note: ***SARS-CoV-2 Spike S1-Biotin*** *is very sensitive to freeze/thaw cycles. Do not re-use thawed aliquots or diluted enzyme.*

4) Dilute **SARS-CoV-2 Spike S1-Biotin** to 5 ng/µl (approximately 50 nM) in **1x Immuno Buffer 1**. Keep diluted protein on ice until use. Discard any unused diluted protein after use.

5) Add another 20 µl of **1x Immuno Buffer 1** to the wells designated "Blank".

	Blank	Positive Control	Test Inhibitor
1x Immuno Buffer 1	40 µl	20 µl	20 µl
Test Inhibitor	-	-	10 µl
Inhibitor buffer (no inhibitor)	10 µl	10 µl	-
SARS-CoV-2 Spike S1-Biotin (5 ng/µl)	-	20 µl	20 µl
Total	**50 µl**	**50 µl**	**50 µl**

6) Initiate reaction by adding 20 µl of diluted **SARS-CoV-2 Spike S1-Biotin** (see Step 1-4) to wells labeled "Positive Control" and "Test Inhibitor". Incubate at room temperature for one hour with slow shaking.

7) Decant to remove supernatant. Wash the plate 3 times with 100 µl/well **1x Immuno Buffer 1**. Tap plate onto clean paper towels to remove liquid.

8) Block wells by adding 100 µl of **Blocking Buffer 2** to each well. Incubate for 10 minutes at room temperature. Remove supernatant as in Step 1-7.

Step 2:

1) Dilute **Streptavidin-HRP** 1000-fold with **Blocking Buffer 2**.

2) Add 100 µl to each well. Incubate for 1 hour at room temperature with slow shaking.

3) Wash plate three times with **1x Immuno Buffer 1**. Tap plate onto clean paper towel to remove liquid.

4) Block wells by adding 100 µl of **Blocking Buffer 2** to each well. Incubate for 10 minutes at room temperature. Decant to remove supernatant. Tap plate onto clean paper towels to remove liquid.

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5) Just before use, mix 50 µl **ELISA ECL Substrate A** and 50 µl **ELISA ECL Substrate B**, then add 100 µl to each well. Discard any unused chemiluminescent reagent after use.

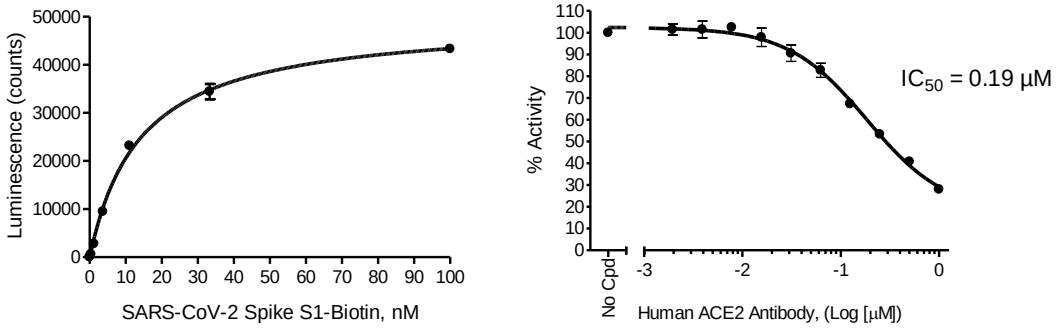
6) Immediately read sample in a luminometer or microtiter-plate capable of reading chemiluminescence. "Blank" value is subtracted from all readings.

Reading Chemiluminescence:

Chemiluminescence is the emission of light (luminescence) which results from a chemical reaction. The detection of chemiluminescence requires no wavelength selection because the method used is emission photometry and is not emission spectrophotometry.

To properly read chemiluminescence, make sure the plate reader is set for LUMINESCENCE mode. Typical integration time is 1 second; delay after plate movement is 100 milliseconds. Do not use a filter when measuring light emission. Typical settings for the Synergy 2 BioTek plate reader are: use the "hole" position on the filter wheel; Optics position: Top; Read type: endpoint. Sensitivity may be adjusted based on the luminescence of a control assay without enzyme (typically we set this value as 100).

Example of assay results:



Binding of SARS-CoV-2 Spike S1-Biotin (BPS Bioscience, #100679) to immobilized ACE2-His (BPS Bioscience, #11003) (left) and inhibition of SARS-CoV-2 Spike S1:ACE2 binding using the Human ACE2 Antibody (R&D Systems, #AF933) (right) in the ACE2:SARS-CoV-2 Spike S1 Inhibitor Screening Assay Kit (BPS Bioscience, #79945). Luminescence was measured using a BioTek microplate reader. Data shown is lot-specific. For lot-specific information, please contact BPS Bioscience, Inc. at info@bpsbioscience.com.

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RELATED PRODUCTS:

Product Name	Catalog#	Size
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-His	11003	100 µg
ACE2 Inhibitor Screening Assay Kit	79923	96 reactions
SARS-CoV-2 Spike S1, Fc Fusion, Avi-tag, Biotin-labeled	100679	25, 50 µg
SARS-CoV-2 Spike RBD, His-tag	100687	50, 100 µg
SARS-CoV-2 Spike S1, Fc-fusion (IgG1)	100688	20, 50 µg
SARS-CoV-2 Spike RBD, Fc-fusion (IgG1)	100699	50, 100 µg
Immuno Buffer 1	79311	50 ml
Blocking Buffer 2	79728	50 ml
ELISA ECL Substrate	79760-1	200 ml

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TROUBLESHOOTING GUIDE

Problem	Possible cause	Solution
Luminescence signal of positive control reaction is weak	SARS-CoV-2 Spike S1-Biotin ACE2-His has lost activity	Proteins lose activity upon repeated freeze/thaw cycles. Use fresh ACE2-His (BPS Bioscience #11003) and fresh SARS-CoV-2 Spike S1-Biotin (BPS Bioscience #100679). Store proteins in single-use aliquots. Increase time of enzyme incubation. Increase enzyme concentration.
	Incorrect settings on instruments	Refer to instrument instructions for settings to increase sensitivity of light detection.
	Chemiluminescent reagents mixed too soon	Chemiluminescent solution should be used within 15 minutes of mixing. Ensure both reagents are properly mixed.
Luminescent signal is erratic or varies widely among wells	Inaccurate pipetting/technique	Run duplicates of all reactions. Use a multichannel pipettor. Use master mixes to minimize errors.
	Bubbles in wells	Pipette slowly to avoid bubble formation. Tap plate lightly to disperse bubbles; be careful not to splash between wells.
Background (signal to noise ratio) is high	Insufficient washes	Increase number of washes. Increase wash volume.
	Sample solvent is inhibiting the enzyme	Run negative control assay including solvent. Maintain DMSO level at <1% Increase time of enzyme incubation.
	Results are outside the linear range of the assay	Use different concentrations of SARS-CoV-2 Spike S1-Biotin (BPS Bioscience, #100679) to create a standard curve