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Data sheet
Spike S1 (SARS-CoV-2): ACE2 Inhibitor Screening Colorimetric Assay Kit

Catalog #79954
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 Inhibitor Screening Colorimetric Assay Kit is designed for screening and profiling inhibitors of this interaction. This kit comes in a convenient 96-well format, with purified SARS-CoV-2 Spike S1 and ACE2-Biotin proteins, streptavidin-HRP, and assay buffer for 100 binding reactions. The key to this kit is the high sensitivity of detection of ACE2-Biotin protein by Streptavidin-HRP. Only a few simple steps on a microtiter plate are required for the assay. First, Spike S1 protein is attached to a 96-well transparent plate. Next, ACE2-Biotin is incubated with Spike S1 on the plate. Finally, the plate is treated with streptavidin-HRP followed by addition of an HRP substrate to produce color, which can then be measured using a UV/Vis spectrophotometer microplate reader.

COMPONENTS:

Catalog #	Component	Amount	Storage	
100678	Spike S1, Fc Fusion, Avi-tag (SARS-CoV-2)	2 µg	-80°C	Avoid multiple freeze/ thaw cycles!
100665	ACE2, His-Avi-Tag, Biotin-labeled HiP™	2 µg	-80°C	
-	Streptavidin-HRP	10 µl	-20°C	
79311	3x Immuno Buffer 1	50 ml	-20°C	
79728	Blocking Buffer 2	50 ml	+4°C	
-	Colorimetric HRP substrate	10 ml	-20°C	
-	Transparent 96-well white microplate	1	Room Temp	

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MATERIALS OR INSTRUMENTS REQUIRED BUT NOT SUPPLIED:

PBS (Phosphate buffered saline)
1N HCl (aqueous)
Rotating or rocker platform
UV/Vis spectrophotometer microplate reader capable of reading absorbance at 450 nm*
**Alternatively, a spectrophotometer reading at 650 nm may be used, but sensitivity of the assay will be greatly reduced.*

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

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.

ASSAY PROTOCOL:

All samples and controls should be tested in duplicate.

Coating the plate with Spike S1:

1) Thaw **Spike S1** on ice. Upon first thaw, briefly spin tube containing **Spike S1** to recover the full contents of the tube. Aliquot into single use aliquots. Immediately store remaining **Spike S1** in aliquots at -80°C. Note: **Spike S1** is very sensitive to freeze/thaw cycles. Avoid multiple freeze/thaw cycles.
2) Dilute **Spike S1** to 2 µg/ml in PBS.
3) Add 50 µl of diluted **Spike S1** 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.

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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). Incubate at room temperature for one hour with slow shaking.

   Note: Final DMSO concentration in the assay should be ≤1%. Inhibitor buffer should contain the same concentration of DMSO as the test inhibitor. For example, prepare 100x test compound in DMSO; dilute 1:20 in water to make a 5% DMSO solution. For the control, use 5% DMSO with no inhibitor. Final DMSO concentration in the assay will be 1%.

3) Thaw **ACE2-Biotin** on ice. Upon first thaw, briefly spin tube containing enzyme to recover full contents of the tube. Aliquot **ACE2-Biotin** into single use aliquots. Immediately store remaining undiluted enzyme in aliquots at -80°C. Note: ***ACE2-Biotin*** *is very sensitive to freeze/thaw cycles. Do not re-use thawed aliquots or diluted enzyme.*

4) Dilute **ACE2-Biotin** to 1 ng/µl (approximately 12 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	-
ACE2-Biotin (1 ng/µl)	-	20 µl	20 µl
Total	**50 µl**	**50 µl**	**50 µl**

6) Initiate reaction by adding 20 µl of diluted **ACE2-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.

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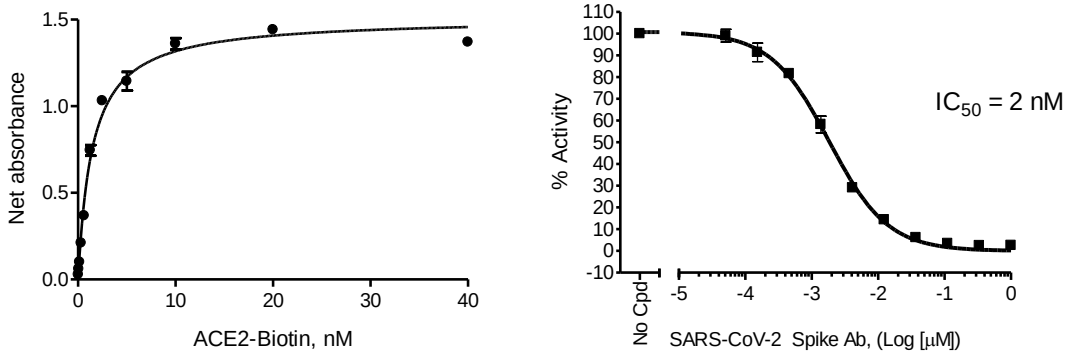
3) Wash plate three times with **1x Immuno Buffer 1**. Tap plate onto clean paper towel to remove liquid.

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.

5) Add 100 µl of the **Colorimetric HRP substrate** to each well and incubate the plate at room temperature until blue color is developed in the positive control well. This usually takes 1-2 min to fully develop. However, the optimal incubation time may vary, and should be determined empirically by the user. Immediately read sample in a luminometer or microtiter-plate capable of reading chemiluminescence. “Blank” value is subtracted from all readings.

6) After the blue color is developed, add 100 µl of 1N HCl to each well. Read the absorbance at 450 nm using UV/Vis spectrophotometer microplate reader. The blank wells should exhibit an absorbance of ~ 0.05 at 450 nm. Alternatively, the plate may be read at 650 nm without adding 1N HCl, but the Signal-to-Background ratio will be decreased.

Example of assay results:



ACE2-Biotin (BPS Bioscience, #10065) binding to immobilized SARS-CoV-2 Spike S1 (BPS Bioscience, #100678) (left) and inhibition of the SARS-CoV-2 Spike S1:ACE2 binding by anti-SARS-CoV-2 Spike Antibody (Active Motif, #91361) (right) using the *ACE2:SARS-CoV-2 Spike Inhibitor Screening Assay Kit*. Luminescence was measured using a Bio-Tek fluorescent 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:SARS-CoV-2 Spike S1-Biotin Inhibitor Screening Assay Kit	79945	96 reactions
SARS-CoV-2 Spike S1-Biotin:ACE2 TR-FRET Assay Kit	79949	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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TROUBLESHOOTING GUIDE:

Problem	Possible cause	Solution
Luminescence signal of positive control reaction is weak	Spike S1 and ACE2-Biotin have lost activity	Proteins lose activity upon repeated freeze/thaw cycles. Use fresh proteins. 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.
	Colorimetric HRP substrate was not incubated long enough	Increase the amount of time that the colorimetric HRP substrate is incubated in the wells. Avoid azides.
Colorimetric 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.
	Signal is out of range of detection (too high)	Decrease the amount of time that the colorimetric HRP substrate is incubated in the 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 proteins to create a standard curve