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SZABO-SCANDIC HandelsgmbH

Quellenstraße 110, A-1100 Wien

T. +43(0)1 489 3961-0

F. +43(0)1 489 3961-7

mail@szabo-scandic.com

www.szabo-scandic.com

linkedin.com/company/szaboscandic

6042 Cornerstone Court W, Ste B
San Diego, CA 92121
Tel: 1.858.202.1401
Fax: 1.858.481.8694
Email: info@bpsbioscience.com

Data Sheet
ACE2: Spike RBD (SARS-CoV-2) Inhibitor Screening Colorimetric Assay Kit

Catalog #78031
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 protein receptor binding domain (RBD) 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 ACE2:SARS-CoV-2 Spike (RBD) 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 ACE2 and SARS-CoV-2 Spike proteins, HRP-labeled anti-mouse Fc region antibody, and assay buffer for 100 binding reactions. The key to this kit is the high sensitivity of detection of Fc-tagged Spike protein by HRP-labeled Anti-mouse-Fc. Only a few simple steps on a microtiter plate are required for the assay. First, ACE2 protein is attached to a clear nickel-coated 96-well plate. Next, SARS-CoV-2 Spike-Fc is incubated with ACE2 on the plate. Finally, the plate is treated with HRP-labeled anti-Fc, followed by addition of an HRP substrate to produce color, which can then be measured using a UV/Vis spectrophotometer microplate reader.

Note: This kit is based on antibody binding to the mouse Fc region. If your sample includes IgG, it may interfere with the assay and create a false positive signal. In this instance, we suggest using the SARS-CoV-2 Spike:ACE2 Inhibitor Screening Colorimetric Assay Kit, #79954.

COMPONENTS:

Catalog #	Component	Amount	Storage	
79932	SARS-CoV-2 Spike Protein (RBD), mFc Tag	10 µg	-80°C	Avoid multiple freeze/ thaw cycles!
11003	ACE2, His-Tag	2 µg	-80°C	
79311	3x Immuno Buffer 1	50 ml	-20°C	
79728	Blocking Buffer 2	50 ml	+4°C	
52130H	Secondary HRP-labeled antibody 1 (mouse)	15 µl	-80°C	
	Colorimetric HRP substrate	10 ml	+4°C	
	Clear nickel-coated 96-well white microplate	1	Room Temp	

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

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

REFERENCES:
Hoffmann, M. *et al. 2020. Cell*, **181(2):**271-280.e1-e5
Yan, R. *et al*. 2020. *Science*, **367(6485):**1444-1448.

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 the sensitivity of the assay will be greatly reduced.*

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 2 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.
6) 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). 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 concentration in the assay should be ≤1%. Inhibitor buffer should contain the same concentration of DMSO as the test inhibitor.

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

4) Dilute **Spike RBD** to 0.5 ng/µl (approximately 10 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	-
Spike RBD (0.5 ng/µl)	-	20 µl	20 µl
Total	**50 µl**	**50 µl**	**50 µl**

6) Initiate reaction by adding 20 µl of diluted **Spike RBD** (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 **Secondary HRP-labeled antibody 1** 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.

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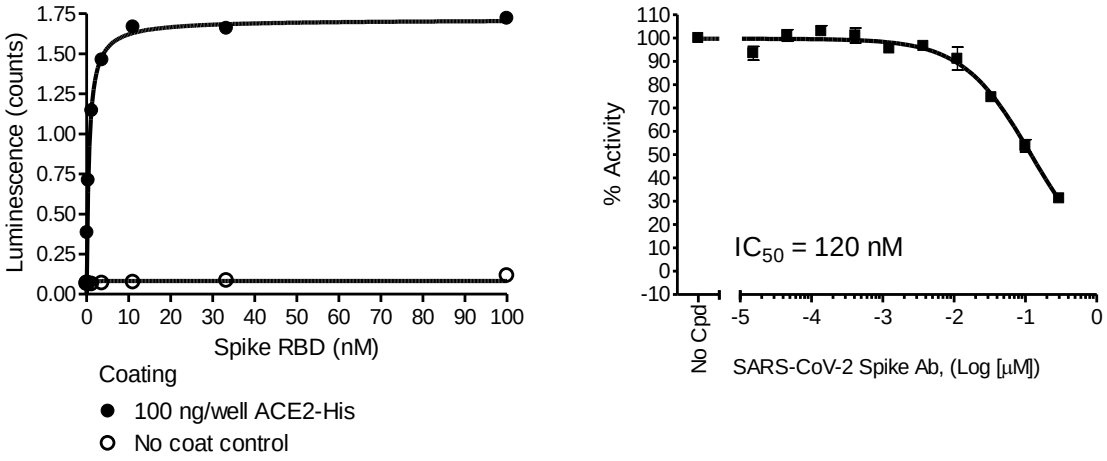
4) Block wells by adding 100 µl of **Blocking Buffer 2** to each well. Incubate for 10 minutes at room temperature. Decant to remove supernatant. Tap plate onto clean paper towels to remove liquid.

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. "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:



Spike RBD (SARS-CoV-2) (BPS Bioscience, #79932) binding to immobilized human ACE2 (BPS Bioscience, #11003) (left) and inhibition of ACE2:Spike RBD (SARS-CoV-2) binding by human anti-SARS-CoV-2 Spike Antibody (BPS Bioscience, #100793) (right) using the *ACE2: Spike RBD (SARS-CoV-2). Inhibitor Screening Colorimetric Assay Kit* (BPS Bioscience, #78031). 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
Spike S1 Neutralizing Antibody (SARS-CoV-2) (Clone: 414-1)	100793	100 µg
SARS-CoV-2 Spike Protein (RBD), mFc Tag	100684	20 µg/50 µg
Immuno Buffer 1	79311	50 ml
Blocking Buffer 2	79728	50 ml
Spike RBD (SARS-CoV-2) : ACE2 Inhibitor Screening Assay Kit	79931	96 reactions
ACE2: Spike RBD (SARS-CoV-2) Inhibitor Screening Assay Kit	79936	96 reactions
ACE2: Spike S1-Biotin (SARS-CoV-2) Inhibitor Screening Assay Kit	79945	96 reactions
Spike S1-Biotin (SARS-CoV-2): ACE2 TR-FRET Assay Kit	79949	96 reactions
Spike S1 (SARS-CoV-2): ACE2 Inhibitor Screening Colorimetric Assay Kit	79954	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
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

TROUBLESHOOTING GUIDE

Problem	Possible cause	Solution
Luminescence signal of positive control reaction is weak	Spike RBD (SARS-CoV-2) or ACE2-His has lost activity	Proteins lose activity upon repeated freeze/thaw cycles. Use fresh ACE2-His (BPS Bioscience #11003) and fresh Spike RBD (SARS-CoV-2) (BPS Bioscience #100684). 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 Spike RBD (SARS-CoV-2) (BPS Bioscience #100684) to create a standard curve