SZABO SCANDIC

Part of Europa Biosite

Produktinformation

- Forschungsprodukte & Biochemikalien
- Zellkultur & Verbrauchsmaterial
- Diagnostik & molekulare Diagnostik
- Laborgeräte & Service

Weitere Information auf den folgenden Seiten!
See the following pages for more information!

Lieferung & Zahlungsart

siehe unsere Liefer- und Versandbedingungen

Zuschläge

- Mindermengenzuschlag
- Trockeneiszuschlag
- Gefahrgutzuschlag
- Expressversand

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:SARS-CoV-2 Spike Inhibitor Screening Assay Kit
Catalog #79936
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 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 proteins, HRP-labeled anti-human 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-Fc. 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-Fc is incubated with ACE2 on the plate. Finally, the plate is treated with Anti-Fc-HRP followed by addition of an HRP substrate to produce chemiluminescence, which then can be measured using a chemiluminescence reader.

Note: This kit is based on binding to the human Fc receptor. If your sample includes human 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 Assay Kit, #79931.

COMPONENTS:

Catalog #	Component	Amount	Storage	
79932	SARS-CoV-2 Spike Protein (RBD), Fc Tag	5 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	
	Anti-Fc-HRP	15 µl	-80°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	

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

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

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. Science*, In Press 4 March 2020. DOI: 10.1126/science.abb2762

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 5% DMSO in water (inhibitor buffer). Incubate at room temperature for one hour with slow shaking.

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

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 **SARS-CoV-2 Spike** on ice. Upon first thaw, briefly spin tube containing enzyme to recover full contents of the tube. Aliquot **SARS-CoV-2 Spike** into single use aliquots. Immediately store remaining undiluted enzyme in aliquots at -80°C. Note: ***SARS-CoV-2 Spike*** *is very sensitive to freeze/thaw cycles. Do not re-use thawed aliquots or diluted enzyme.*
4) Dilute **SARS-CoV-2 Spike** to 1 ng/µl (approximately 20 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
5% DMSO in water (Inhibitor buffer)	10 µl	10 µl	-
SARS-CoV-2 Spike (1 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** (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 **Anti-Fc-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.

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

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) 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.

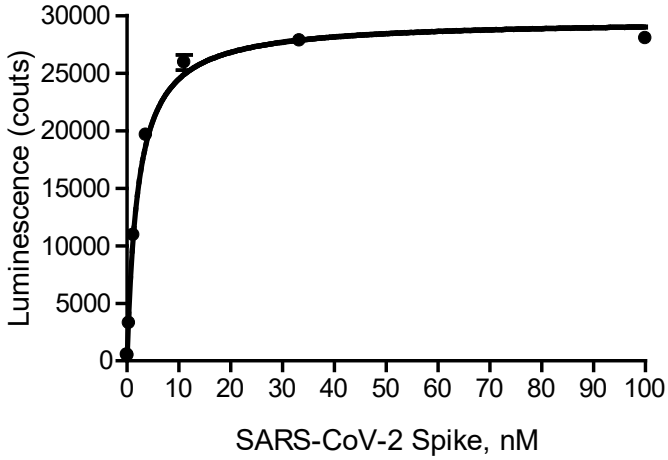
6) Immediately read sample in a luminometer or microtiter-plate capable of reading chemiluminescence. "Blank" value is subtracted from all readings.

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:



SARS-CoV-2 Spike binding to immobilized ACE2 using the *ACE2:SARS-CoV-2 Spike Inhibitor Screening Assay Kit (BPS Bioscience #79936)*. 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.*

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

RELATED PRODUCTS:

Product Name	Catalog#	Size
SARS-CoV-2 Spike:ACE2 Inhibitor Screening Assay Kit	79931	96 reactions
ACE2-His	71158	100 µg
ACE2 Inhibitor Screening Assay Kit	79923	96 reactions
SARS-CoV-2 Spike	TBD	
Immuno Buffer 1	79311	50 ml
Blocking Buffer 2	79728	50 ml
ELISA ECL Substrate	79760-1	200 ml

TROUBLESHOOTING GUIDE

Problem	Possible cause	Solution
Luminescence signal of positive control reaction is weak	SARS-CoV-2 Spike 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 (BPS Bioscience #TBD). 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 (BPS Bioscience #TBD) to create a standard curve