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Produktinformation

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

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See the following pages for more information!

Lieferung & Zahlungsart

siehe unsere Liefer- und Versandbedingungen

Zuschläge

- Mindermengenzuschlag
- Trockeneiszuschlag
- Gefahrgutzuschlag
- Expressversand

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Description

The Spike S1 RBD (B.1.1.529 BA.1, Omicron Variant) (SARS-CoV-2): ACE2 Inhibitor Screening Chemiluminescence Assay Kit is designed for screening and profiling neutralizing antibodies or inhibitors of the interaction between the Omicron variant SARS-CoV-2 Spike RBD and human ACE2. This kit comes in a convenient 96-well format, with Biotinylated-ACE2, purified Spike RBD (Omicron Variant; B.1.1.529 BA.1) protein, Streptavidin-HRP, and assay buffers for 100 reactions.

The assay requires only a few steps. First, SARS-CoV-2 Spike RBD (Omicron Variant; B.1.1.529 BA.1) is coated on a 96-well plate overnight. After washing and blocking, the protein is pre-incubated with an inhibitor or neutralizing antibody. Upon subsequent incubation with Biotin-ACE2, the plate is treated with Streptavidin-HRP followed by addition of a chemiluminescence HRP substrate to produce the luminescence signal.

Background

The COVID-19 pandemic is caused by Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2). The Spike glycoprotein is expressed on the surface of the virus as a trimer. Each Spike protein consists of two subunits, S1 and S2, and the S1 subunit contains the receptor binding domain (RBD) which recognizes and attaches to the ACE2 receptor found on the surface of type I and II pneumocytes, endothelial cells, and ciliated bronchial epithelial cells. SARS-CoV-2 Variant B.1.1.529 BA.1, also known as Omicron variant, was originally discovered in South Africa and has recently become a global variant of concern.

Drugs targeting the interaction between SARS-CoV-2 Spike protein and human ACE2 may offer some protection against viral infection. The SARS-CoV-2 Spike RBD (B.1.1.529 BA.1, Omicron Variant) protein in this kit consists of the RBD region of the S1 protein, which includes the ACE2 binding site. The omicron variant of SARS-CoV-2 has at least 3 sublineages, BA.1., BA.2, and BA.3. The protein contains all the mutations observed in this region of the omicron B.1.1.529 BA.1 variant compared to the wild-type strain.

Applications

This kit is useful for screening for inhibitors of ACE2 binding to the RBD domain of SARS-CoV-2 Spike, Omicron variant.

Supplied Materials

Catalog #	Name	Amount	Storage
	Spike S1 RBD (B.1.1.529 BA.1, Omicron Variant), His-Tag (SARS-CoV-2)*	5 µg	-80°C
100665	ACE2, His-Avi-Tag, Biotin-labeled HiP™	2 x 5 µg	-80°C
79311	3x Immuno Buffer 1	50 ml	-20°C
79728	Blocking Buffer 2	50 ml	+4°C
79742	Streptavidin-HRP	15 µl	+4°C
79670	ELISA ECL Substrate A (translucent bottle)	6 ml	Room Temp
	ELISA ECL Substrate B (brown bottle)	6 ml	Room Temp
79699	White 96-well microplate	1	Room Temp

**The initial concentration of both ACE2 and Spike RBD is lot-specific and will be indicated on the tube containing the protein.*

Materials Required but Not Supplied

PBS (Phosphate buffered saline)
Rotating or rocker platform
Luminescence microplate reader
Neutralizing anti-Spike antibody (as a control)

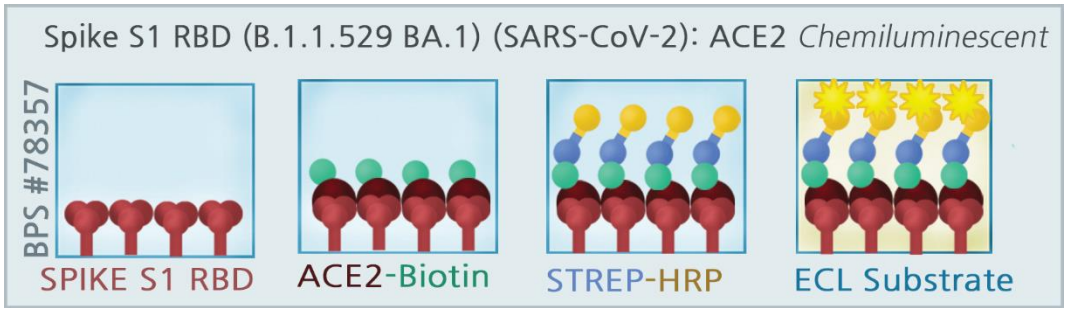
Storage Conditions

This assay kit will perform optimally for up to 6 months from date of receipt when the materials are stored as directed.

Safety

This product is for research purposes only and not for human or therapeutic use. This product should be considered hazardous and is harmful by inhalation, in contact with skin, eyes, clothing, and if swallowed. If contact occurs, wash thoroughly.

Assay Principle



Assay Protocol

All samples and controls should be tested in duplicate. We recommend preincubating antibodies or protein inhibitors with the target protein. For small molecule inhibitors, pre-incubation may also beneficial, depending on the experimental conditions.

Coating the plate with Spike S1 RBD protein overnight and blocking:

1) Thaw **Spike S1 RBD (B.1.1.529 BA.1, Omicron Variant) protein** on ice. Briefly spin tube to recover the full contents of the tube. Note: **Spike protein** is very sensitive to freeze/thaw cycles. Avoid multiple freeze/thaw cycles. Do not re-use a diluted protein.
2) Dilute **Spike S1 RBD protein** to 1 µg/ml in PBS.
3) Add 50 µl of diluted **Spike S1 RBD protein** solution to each well and incubate at 4°C overnight.
4) Prepare **1x Immuno Buffer 1** by diluting **3x Immuno Buffer 1** in sterile distilled water.
5) After the overnight coating, discard the solution and wash the plate three times with 100 µl **1x Immuno Buffer 1**. Tap plate onto clean paper towels to remove excess liquid.

6) Block wells by adding 100 µl **Blocking Buffer 2** to each well. Incubate for 1 hour at room temperature with slow shaking. Remove the blocking solution and tap the plate onto clean paper towels to remove excess liquid.

Step 1

1) Prepare dilutions of neutralizing anti-Spike antibody or test inhibitor in **Blocking Buffer 2** to the desired concentration (it is recommended to use serial dilutions). Prepare enough for 50 µl per well. Note: high concentrations of DMSO may interfere with protein binding. If the test inhibitor compound is dissolved in DMSO, the final DMSO concentration in the assay should be ≤1%.
2) Add 50 µl of the diluted antibody or inhibitor to the wells labeled "Test Inhibitor". To the wells labeled "Blank" and "Positive Control", add 50 µl of **Blocking Buffer 2**. Incubate the plate for 30 minutes (up to 1 hour) at room temperature with slow rotation.
3) Thaw the **Biotin-ACE2** on ice, briefly spin tube to recover the full contents of the tube, and dilute it to 1.5 ng/µl in **Blocking Buffer 2**. Note: **Biotin-ACE2** is very sensitive to freeze/thaw cycles. Avoid multiple freeze/thaw cycles. Do not re-use a diluted protein.
4) Add 50 µl of diluted **Biotin-ACE2** to the wells labeled "Test Inhibitor" and "Positive Control". Add 50 µl **Blocking Buffer 2** to the wells labeled "Blank". Incubate the plate at room temperature for 1 hour with slow rotation.

Component	Blank	Positive Control	Test Inhibitor
1x Immuno Buffer 1	100 µl	50 µl	-
Test antibody or inhibitor	-	-	50 µl
ACE2-Biotin (1.5 ng/µl)	-	50 µl	50 µl
Total	100 µl	100 µl	100

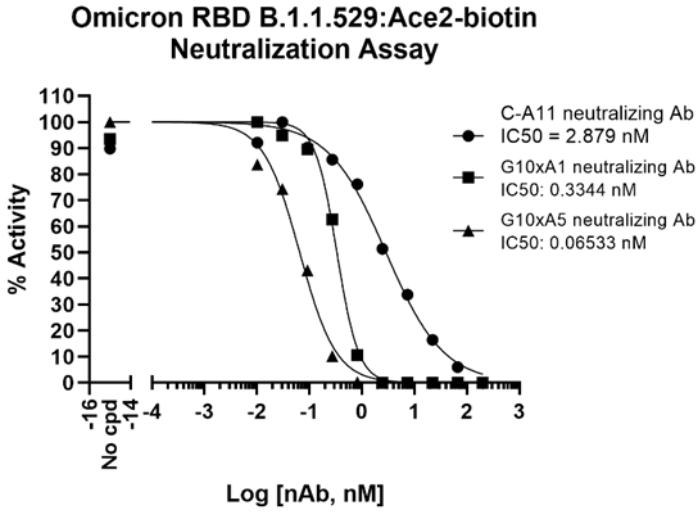
5) After 1 hour, discard the solution and wash the plate three times with 100 µl **1x Immuno Buffer 1**. Tap plate onto clean paper towels to remove excess liquid.
6) Block by adding 100 µl **Blocking Buffer 2** to each well. Incubate for 10 minutes at room temperature. Discard the solution and tap plate onto clean paper towels to remove excess liquid.

Step 2

1) Dilute **Streptavidin-HRP** 1000-fold using **Blocking Buffer 2.**
2) Add 50 µl of the **diluted Streptavidin-HRP** to each well and incubate the plate for 1 hour at room temperature with slow shaking.
3) After 1 hour, discard the solution and wash the plate three times with 100 µl **1x Immuno Buffer 1**. Tap plate onto clean paper towels to remove excess liquid.
4) Block by adding 100 µl of **Blocking Buffer 2** to each well. Incubate for 10 minutes at room temperature. Discard the solution and tap plate onto clean paper towels to remove excess liquid.
5) Just before use, mix 50 µl of **ELISA ECL substrate A** and 50 µl of **ELISA ECL substrate B** per well, then add 100 µl to each well. Discard any unused chemiluminescent reagent after use.
6) Immediately read in a luminometer or microtiter-plate capable of reading chemiluminescence.

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 msec. 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 signal strength.

Example Results



Inhibition of ACE2: Spike S1 RBD (B.1.1.529 BA.1, Omicron Variant) binding by three anti-SARS-CoV-2 Spike antibodies. Anti-SARS-CoV-2 Spike antibodies C-A11 (BPS Bioscience, #101024), G10xA1 (BPS Bioscience, #101326) and G10xA5 (BPS Bioscience, #101327) were evaluated using the ***Spike S1 RBD (B.1.1.529 BA.1, Omicron Variant) (SARS-CoV-2): ACE2 Inhibitor Screening Chemiluminescence Assay Kit***. The antibodies were serially diluted in Blocking Buffer 2 (using serial dilutions from 0 to 200 nM) and tested following the assay kit protocol.

General considerations

"Blank" Control: The "Blank" control is important to determine the background absorbance in the assay.

Troubleshooting Guide

Problem	Possible Causes	Recommended Solutions
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.
	Sample solvent is inhibiting the enzyme	Run negative control assay including solvent. Maintain DMSO level at <1% Increase time of enzyme incubation.
	Incorrect settings on instruments	Refer to instrument instructions for correct settings to increase sensitivity of light detection.
Luminescence 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.
	Results are outside the linear range of the assay	Use different concentrations of proteins to create a standard curve

Visit bpsbioscience.com/assay-kits-faq for detailed troubleshooting instructions. For all further questions, please email support@bpsbioscience.com

Related Products

Products	*Catalog #*	*Size*
Spike S1 RBD (B.1.1.529 BA.1, Omicron Variant) (SARS-CoV-2): ACE2 Inhibitor Screening Colorimetric Assay Kit	78339	96 reactions
Spike Neutralizing Antibody (Clone G10xA1) (SARS-CoV-2)	101326	100 µg
Spike Neutralizing Antibody (Clone G10xA5) (SARS-CoV-2)	101327	100 µg
Spike S1 Neutralizing Antibody (Clone C-A11) (SARS-CoV-2)	101024	100 µg
Spike S1 RBD (SARS-CoV-2): ACE2 Inhibitor Screening Assay Kit	79931	96 reactions
ACE2: Spike S1 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	96/384 reactions
ACE2, His-Avi-Tag	11003-1	20 µg
ACE2, His-Avi-Tag, Biotin-labeled HiP™	100665-1	20 µg
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