Description

The Spike Trimer (S1+S2) (B.1.1.529 BA.1, Omicron Variant) (SARS-CoV-2): ACE2 Inhibitor Screening Chemiluminescence Assay Kit is designed for screening and profiling inhibitors or neutralizing antibodies of the interaction between the SARS-CoV-2 Omicron variant Spike Trimer and human ACE2. This kit comes in a convenient 96-well format, with Biotinylated-ACE2, purified Spike Trimer protein (B.1.1.529 BA.1, Omicron Variant), Streptavidin-HRP, and assay buffers for 100 reactions. The SARS-CoV-2 Spike Trimer, included in the kit, provides a biologically relevant model for the investigation of SARS-CoV-2/ACE2 interaction.

The assay requires only a few steps. First, SARS-CoV-2 Spike Trimer (B.1.1.529 BA.1, Omicron Variant) 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 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. This variant contains a number of mutations that increase infectivity and transmissibility.

Drugs targeting the interaction between SARS-CoV-2 Spike protein and human ACE2 may offer some protection against viral infection. This kit includes the **SARS-CoV-2 Spike Trimer (B.1.1.529 BA.1, Omicron Variant) protein** in its native trimeric conformation to provide a relevant screen for inhibitors of the Spike S1:ACE2 interaction.

Applications

This kit is useful for screening inhibitors of ACE2 binding to **SARS-CoV-2 Spike Trimer (S1+S2) (B.1.1.529 BA.1, Omicron Variant)**

Supplied Materials

Catalog #	Name	Amount	Storage
101343	Spike Trimer (S1+S2) (B.1.1.529 BA.1, Omicron Variant), His-Tag (SARS-CoV-2)*	20 µg	-80°C
100665	ACE2, 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	10 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 Trimer is lot-specific and will be indicated on the tube containing the protein.*

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Materials Required but Not Supplied

Name
PBS (Phosphate buffered saline)
PBST (0.05% Tween-20)
Rotating or rocker platform
Luminescence microplate reader

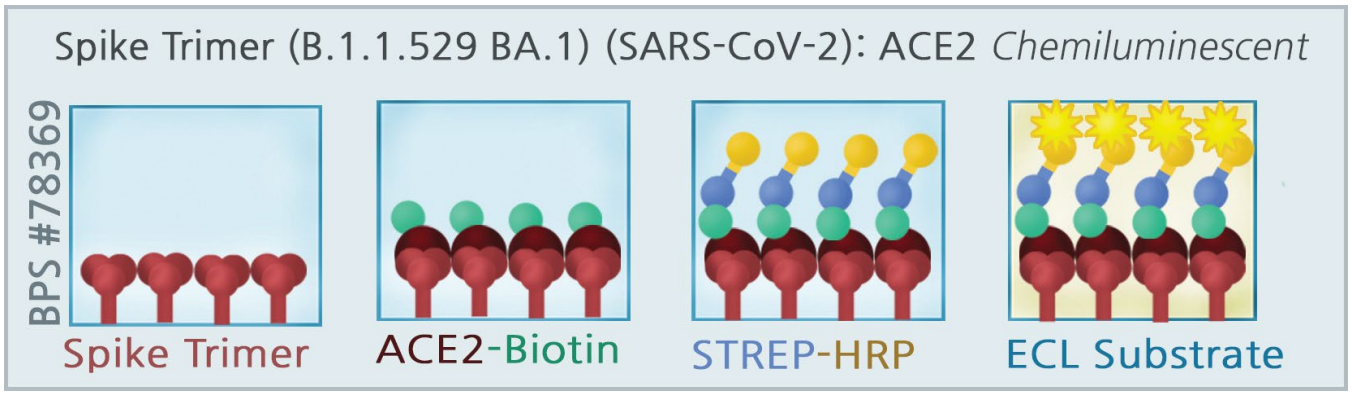
Storage Conditions

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:



Contraindications

DMSO concentration in the final reaction should be ≤1%.

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Assay Protocol
All samples and controls should be tested in duplicate.

Day 1- Coating the plate with Spike Trimer protein overnight:

1) Thaw **Spike Trimer (B.1.1.529 BA.1, Omicron Variant) protein** on ice. Briefly spin the tube to recover its full contents. 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 Trimer protein** to 4 µg/ml in PBS.
3) Add 50 µl of diluted **Spike Trimer protein** solution to each well. Incubate at +4°C overnight.

Day 2 - Blocking

4) After the overnight coating, discard the solution and wash the plate three times with 100 µl PBST. Tap the plate onto clean paper towels to remove the excess liquid.
5) Block wells by adding 100 µl of **Blocking Buffer 2** to each well. Incubate for 1 hour at room temperature with slow shaking. Remove the blocking solution and wash three times with 100 µl of PBST. Tap the plate onto clean paper towels to remove the excess liquid.

Step 1

1) Prepare **1x Immuno Buffer 1** by diluting **3x Immuno Buffer 1** in sterile distilled water.
2) Add 25 µl of **1x Immuno Buffer 1** to all wells.
3) Prepare dilutions of neutralizing anti-Spike antibody or test inhibitor in **1x Immuno Buffer 1** at concentrations 10-fold higher than the desired final concentrations (it is recommended to use serial dilutions). Prepare enough for 5 µl per well.

   Note: high concentrations of DMSO may interfere with protein binding. If the test inhibitor is dissolved in DMSO, the final DMSO concentration in the assay should be ≤1%.
4) Add 5 µl of the diluted antibody or inhibitor to the wells labeled "Test Inhibitor." To the wells labeled "Blank" and "Positive Control," add 5 µl of **1x Immuno Buffer 1**.
5) Incubate the plate for 30 minutes at room temperature with slow shaking.
6) Meanwhile, thaw the **ACE2[B]** on ice, briefly spin to recover the full contents of the tube, and dilute it to 3.75 ng/µl in **1x Immuno Buffer 1**. Note: **ACE2[B]** is very sensitive to freeze/thaw cycles. Avoid multiple freeze/thaw cycles. Do not re-use a diluted protein.
7) Add 20 µl of diluted **ACE2[B]** to the wells labeled "Test Inhibitor" and "Positive Control." Add 20 µl **1x Immuno Buffer 1** to the wells labeled "Blank." Incubate the plate at room temperature for 1 hour with slow shaking.

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Components	Blank	Positive Control	Test Inhibitor
1x Immuno Buffer 1	50 µl	30 µl	25 µl
Test antibody or inhibitor	-	-	5 µl
ACE2-Biotin (3.75 ng/µl)	-	20 µl	20 µl
Total	**50 µl**	**50 µl**	**50 µl**

8) After 1 hour, discard the solution and wash the plate three times with 100 µl of **1x Immuno Buffer 1**. Tap the plate onto clean paper towels to remove the excess liquid.

9) Block by adding 100 µl of Blocking Buffer 2 to each well. Incubate for 10 minutes at room temperature. Remove the blocking solution and wash three times with 100 µl of **1x Immuno Buffer 1**. Tap the plate onto clean paper towels to remove the excess liquid.

Step 2

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

2) Add 100 µl of the diluted **Streptavidin-HRP** to each well and incubate the plate for 30 minutes at room temperature with slow shaking.

3) After 30 minutes, discard the solution and wash the plate three times with 100 µl of **1x Immuno Buffer 1**. Tap the plate onto clean paper towels to remove the excess liquid.

4) Block by adding 100 µl of Blocking Buffer 2 to each well. Incubate for 10 minutes at room temperature. Remove the blocking solution and wash three times with 100 µl of **1x Immuno Buffer 1**. Tap the plate onto clean paper towels to remove the 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. Subtract "blank" value from all other values.

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.

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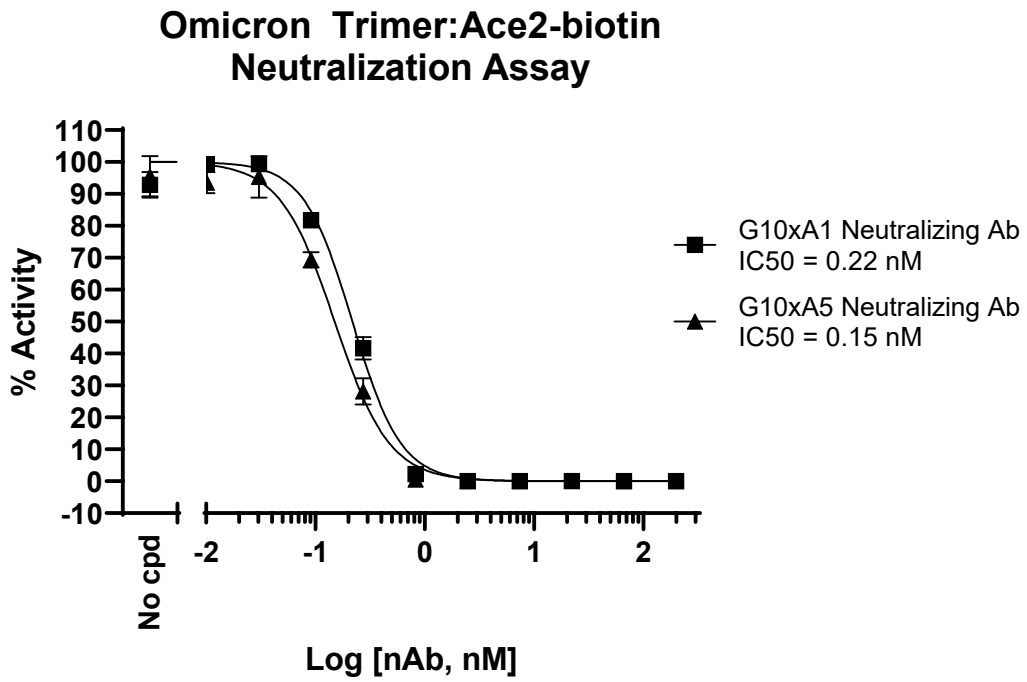
Example Results



*Inhibition of ACE2: SARS-CoV-2 Spike Trimer (B.1.1.529 BA.1, Omicron Variant) binding by two anti-SARS-CoV-2 Spike neutralizing antibodies***.** Anti-Spike neutralizing antibodies G10xA1 (BPS Bioscience #101326) and G10xA5 (BPS Bioscience #101327) were evaluated using the Spike Trimer (S1+S2) (B.1.1.529 BA.1, Omicron Variant) (SARS-CoV-2): ACE2 Inhibitor Screening Chemiluminescence Assay Kit following the assay protocol. The antibodies were serially diluted from 200 nM in 3-fold dilutions.

Data shown is representative. For lot-specific information, please contact BPS Bioscience at support@bpsbioscience.com

General Considerations

"Blank" Control: The "Blank" control is important to determine the background absorbance in the assay. We recommend doing these in duplicate.

Troubleshooting Guide

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

References:

Hoffman M. *et al*., SARS-CoV-2 Cell Entry Depends on ACE2 and TMPRSS2 and Is Blocked by a Clinically Proven Protease Inhibitor. *Cell* 2020; **181:**1-10.

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Related Products

Products	*Catalog #*	*Size*
Spike Neutralizing Antibody (Clone G10xA1) (SARS-CoV-2)	101326	100 µg
Spike Neutralizing Antibody (Clone G10xA5) (SARS-CoV-2)	101327	100 µg
Spike Trimer (S1+S2) (B.1.1.529 BA.1, Omicron Variant) (SARS-CoV-2): ACE2 Inhibitor Screening Colorimetric Assay Kit	78365	96 reactions
Spike S1 RBD (B.1.1.529 BA.1, Omicron Variant) (SARS-CoV-2): ACE2 Inhibitor Screening Colorimetric Assay Kit	78339	96 reactions
Spike S1 RBD (B.1.617.2, Delta Variant), Avi-His-Tag (SARS-CoV-2) HiP™	101153	100 µg/1 mg
Spike Trimer (S1+S2) (B.1.617.2; Delta Variant), His-Tag (SARS-CoV-2)	101147	100 µg
Spike Trimer (S1+S2) (B.1.617.2.1, Delta Plus Variant), His-Tag (SARS-CoV-2)	101165	100 µg
ACE2, His-Avi-Tag, Biotin-labeled HiP™	100665	20 µg/50 µg
Spike Trimer (S1+S2) (B.1.1.7, Alpha Variant), His-Tag (SARS-CoV-2)	510334	100 µg/1 mg
Spike Trimer (S1+S2), His-tag (SARS-CoV-2)	100728	100 µg/1 mg
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 reactions
Spike S1 (13-665), Fc Fusion, Avi-tag (SARS-CoV-2)	100678	100 µg/1 mg
Spike S1 (13-665), 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 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

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