

### Description

The Spike Trimer (S1+S2) (B.1.1.529 BA.1, Omicron Variant) (SARS-CoV-2): ACE2 Inhibitor Screening Colorimetric 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 (B.1.1.529 BA.1, Omicron Variant) protein (His-tagged), 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/host cell 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 a colorimetric HRP substrate to produce color, which can be measured using a UV/Vis microplate reader.

### 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 physiologically 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, His-Avi-Tag, Biotin labeled*	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
79651	Colorimetric HRP substrate	10 ml	+4°C
	Transparent 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.

### Materials Required but Not Supplied

Name	Catalog #
PBS (Phosphate buffered saline)	
1N HCl (aqueous)	
Rotating or rocker platform	
UV/Vis spectrophotometer microplate reader capable of reading absorbance at 450 nm*	

### Storage Conditions



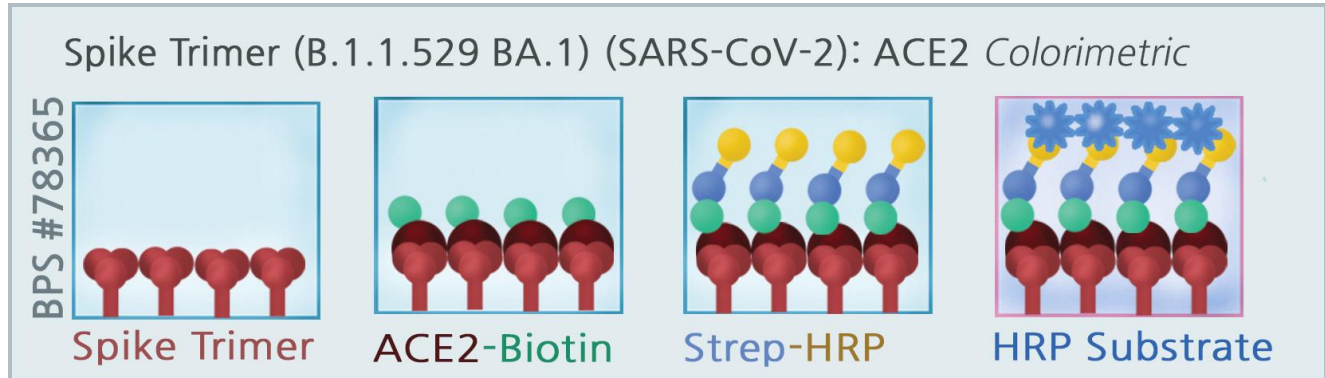
This assay kit will perform optimally for up to 6 months from date of receipt when the materials are stored as directed.

### 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  $\leq 1\%$ .

## Assay Protocol:

### Inhibition of Spike Trimer (B.1.1.529 BA.1, Omicron Variant) binding to ACE2 using an anti-SARS-CoV-2 Spike antibody or inhibitor

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) 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**.
- 3) Incubate the plate for 30 minutes (up to 1 hour) at room temperature with slow rotation.
- 4) Meanwhile, thaw the **Biotin-ACE2** on ice, briefly spin 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.
- 5) 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 agitation.

-	<u>Blank</u>	<u>Positive Control</u>	<u>Test Inhibitor</u>
Blocking Buffer 2	100 µl	50 µl	-
Test antibody or inhibitor	-	-	50 µl
ACE2-Biotin (1.5 ng/µl)	-	50 µl	50 µl
<b>Total</b>	<b>100 µl</b>	<b>100 µl</b>	<b>100 µl</b>

- 6) 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.

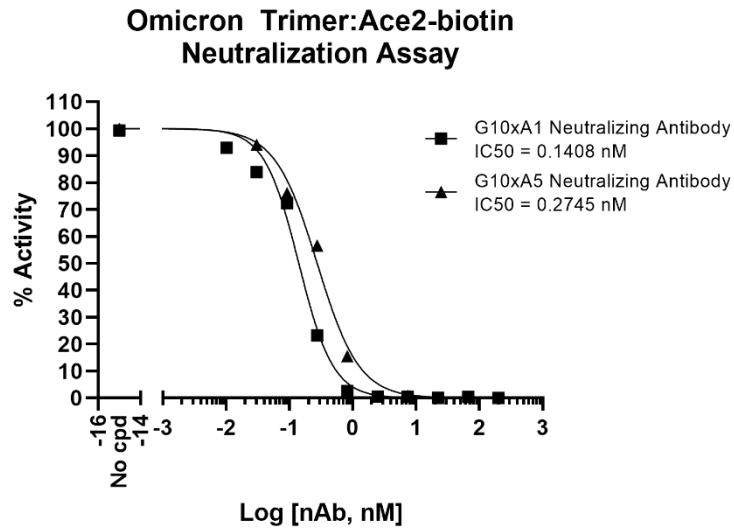
## Step 2

- 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 30 minutes to 1 hour at room temperature with slow shaking.
- 3) Meanwhile, prepare enough 1M HCl (aqueous-stop solution) for 50 µl per well.

Note: alternatively, 2N H<sub>2</sub>SO<sub>4</sub> or other compatible acidic solutions can be substituted.

- 4) 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.
- 5) Add 50 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' wells. This usually takes 1-5 minutes. The optimal incubation time may vary, and should be determined empirically by the user. It is recommended that the reaction be stopped when the 'Positive Control' well is lower than ~ 1.0 absorbance at 450 nm (preferably ~ 0.6).
- 6) Once a blue color has developed in the 'Positive Control' well, add 100 µl of HCl stop solution prepared above to every well. The blue color should turn yellow.
- 7) Read the absorbance at 450 nm using UV/Vis spectrophotometer microplate reader.

## Example Results



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

Data shown is representative. For lot-specific information, please contact BPS Bioscience at [support@bpsbioscience.com](mailto: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](https://bpsbioscience.com/assay-kits-faq) for detailed troubleshooting instructions. For all further questions, please email [support@bpsbioscience.com](mailto: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.

## Related Products

<i>Products</i>	<i>Catalog #</i>	<i>Size</i>
Spike S1 Neutralizing Antibody (Clone G10xA1) (SARS-CoV-2)	101326	100 µg
Spike S1 Neutralizing Antibody (Clone G10xA5) (SARS-CoV-2)	101327	100 µg
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.1.351 Variant), His-Tag (SARS-CoV-2)	510334	100 µg/1 mg
Spike Trimer (S1+S2), His-tag (SARS-CoV)	100789	100 µg/500 µg
Spike Trimer (S1+S2), His-tag (HCoV-NL63)	100788	100 µg/500 µg
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	96/384 reactions
ACE2, His-Avi-Tag		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
ACE2 Inhibitor Screening Assay Kit	79923	96 reactions
ACE2, His-tag	11003	20 µg/100 µg