Description

The Spike Trimer (S1+S2) (P.1; Gamma Variant) (SARS-CoV-2): ACE2 Inhibitor Screening Colorimetric Assay Kit is designed for screening and profiling inhibitors or neutralizing antibodies of the interaction between SARS-CoV-2 Spike and human ACE2. This kit comes in a convenient 96-well format, with Biotinylated-ACE2, purified Spike Trimer (S1+S2) (P.1 Variant) protein (His-tagged), Streptavidin-HRP, and assay buffers for 100 reactions. The key to this kit is that the Spike Trimer (S1+S2) (P.1 Variant) (SARS-CoV-2) protein provides a biologically relevant model for the investigation of SARS-CoV-2/host cell interaction.

The assay requires only a few steps. First, Spike Trimer (S1+S2) (Gamma Variant) (SARS-CoV-2) is coated on a 96-well plate overnight. After blocking, the protein is pre-incubated with the 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 quenched and 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. The P.1 variant of the SARS-CoV-2 Spike protein (also known as Gamma variant) was originally discovered in Brazil and contains 11 mutations (L18F, T20N, P26S, D138Y, R190S, K417T, E484K, N501Y, D614G, H655Y, T1027I). Drugs targeting the interaction between SARS-CoV-2 Spike and human ACE2 may offer some protection against viral infection. This kit includes the **Spike Trimer (S1+S2) (P.1; Gamma Variant) (SARS-CoV-2)** (BPS Bioscience #100989) 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 **Spike Trimer (S1+S2) (P.1 Variant) (SARS-CoV-2)**

Supplied Materials

Catalog #	Name	Amount	Storage
100989	Spike Trimer (S1+S2) (P.1 Variant), His-Tag (SARS-CoV-2) *	5 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	5 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.

Contraindications

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

Assay Protocol

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

Day 1-Coating the plate with SARS-CoV-2 Spike Trimer protein:

1) Thaw **Spike Trimer (S1+S2) (P.1; Gamma Variant) protein** on ice. Upon first thaw, briefly spin the tube to recover the full contents. Aliquot into single use aliquots. Immediately store the remaining in aliquots at -80°C.

   Note: **Spike protein** is very sensitive to freeze/thaw cycles. Avoid multiple freeze/thaw cycles.

2) Dilute **Spike Trimer protein** to 1 µg/ml in PBS.

3) Add 50 µl of diluted **Spike Trimer protein** solution to each well and incubate at 4°C overnight.

Day 2:

1) Prepare **1x Immuno Buffer** by diluting **3x Immuno Buffer** in distilled water.

2) After the overnight coating, discard the solution by flipping the plate over waste container or sink, then tap the plate onto paper towels. Wash the plate three times with 100 µl of **1x Immuno Buffer 1** per well. Tap plate onto clean paper towels to remove liquid.

3) Block 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 tap to dry.

****Note there are two methods for steps 4-9 depending on your inhibitor****

If testing an anti-Spike antibody as inhibitor, follow Steps 4-8 below:

4) Prepare dilutions of neutralizing anti-Spike antibody in **Blocking Buffer 2** to desired concentration (it is recommended to use serial dilutions). Prepare enough for 50 uL per well.

5) Add 50 µl of the diluted antibody to the "Test Inhibitor" wells. To wells designated "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.

6) Meanwhile, thaw the **Biotin-ACE2** on ice, and dilute it to 1.5 ng/µl in **Blocking Buffer 2**. Prepare only the amount required for the assay (50 ul/well); store the remaining **Biotin-ACE2** undiluted at -80°C.

Note: Biotin-ACE2 is very sensitive to freeze/thaw cycles. Avoid multiple freeze/thaw cycles.

7) After the antibody incubation, add an equal volume (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". At this step, there should be a total of 100 µl in each well. Incubate the plate at room temperature for another 1 hour with slow rotation.

	Blank	**Positive Control**	**Test Inhibitor**
Blocking Buffer 2	100 µl	50 µl	-
Test antibody	-	-	50 µl
ACE2-Biotin (1.5 ng/µl)	-	50 µl	50 µl
Total	**100 µl**	**100 µl**	**100 µl**

8) After 1 hour, discard the solution and wash the plate three times with 1x Immuno Buffer 1

If testing a small molecule inhibitor, follow steps 4-8 below:

4) Prepare the test inhibitor in DMSO (or distilled water if soluble in aqueous solution), and further dilute it in distilled water at 10X testing concentration. (*e.g.* To test a compound at 10 µM, prepare the inhibitor in DMSO at 1 mM. Then make a 10-fold dilution in distilled water to obtain a 100 µM solution in 10% DMSO).

5) Add 5 µl to each well labeled "Test Inhibitor". To the "Positive Control" and "Blank" wells, add 5 µl of the diluent solution without inhibitor (e.g. 10% DMSO solution in water) so that all wells contain the same amount of DMSO. *Caution! – It is highly recommended that the final DMSO concentration should not*

exceed 1%. Organic solvents other than DMSO have not been validated in this assay, so use of these solvents must be optimized by the user.

6) Thaw the **Biotin-ACE2** on ice, and dilute it in **Blocking Buffer 2** at 1.5 ng/µl. Prepare only the amount required for the assay; store the remaining **Biotin-ACE2** undiluted at -80°C.

Note: Biotin-ACE2 is very sensitive to freeze/thaw cycles. Avoid multiple freeze/thaw cycles.

7) Add 20 µl of **Blocking Buffer 2** to the wells labeled "Blank". Add 20 µl of diluted **Biotin-ACE2** to the wells labeled "Test Inhibitor" and "Positive Control". Incubate the plate at room temperature for 1 hour with slow rotation.

	Blank	**Positive Control**	**Test Inhibitor**
Blocking Buffer 2	45 µl	25 µl	25 µl
Test Inhibitor	-	-	5 µl
Diluent solution (no inhibitor) –usually 10% DMSO in water	5 µl	5 µl	-
ACE2-Biotin (1.5 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 **Blocking Buffer 2**. Tap the plate onto clean paper towels to remove liquid.

Day 2-Detection:

9) Dilute **Streptavidin-HRP** 1000-fold with the **Blocking Buffer 2**, enough for 50 µl per well.

10) Add 50 µl of the **diluted Streptavidin-HRP** to each well and incubate the plate for 30 minutes at room temperature with slow rotation.

11) After 30 minutes, discard the solution and wash the plate three times.

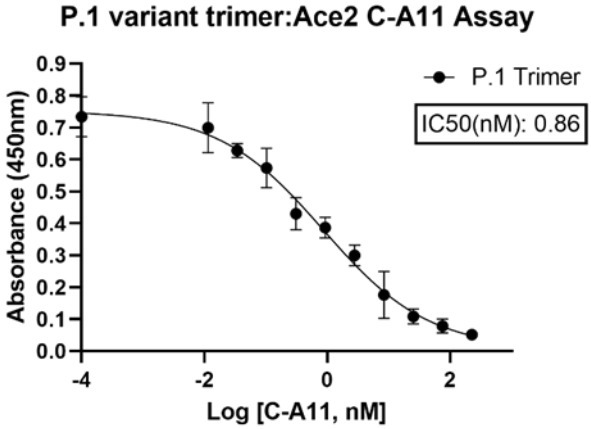
12) Meanwhile, prepare enough 1M HCl (aqueous-stop solution) for 100 µl per well. *Note: alternatively, 2N H_2SO_4 or other compatible acidic solutions can be substituted.*

13) 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' 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).

14) 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.

15) Read the absorbance at 450 nm using UV/Vis spectrophotometer microplate reader.

Example Results



Inhibition of ACE2: Spike Trimer (P.1; Gamma Variant) (SARS-CoV-2) binding by an anti-SARS-CoV-2 Spike neutralizing antibody. C-A11, an anti-SARS-CoV-2 Spike antibody (BPS cat#101024) was evaluated using the Spike Trimer (P.1; Gamma Variant) (SARS-CoV-2): ACE2 Inhibitor Screening Colorimetric Assay Kit. The antibody was serially diluted from 100 nM in 3-fold dilutions and tested following the assay kit protocol. Data shown is representative. For lot-specific information, please contact BPS Bioscience, Inc. 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.

Related Products

Products	*Catalog #*	*Size*
Spike S1 Neutralizing Antibody (Clone C-A11) (SARS-CoV-2)	101024	100 µg
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 S1 (B.1.1.7, Alpha Variant) (SARS-CoV-2): ACE2 Inhibitor Screening Colorimetric Assay Kit	78155	96 reactions
Spike S1 RBD (B.1.351, Beta Variant) (SARS-CoV-2): ACE2 Inhibitor Screening Colorimetric Assay Kit	78152	96 reactions
Spike Trimer (S1+S2) (B.1.1.7, Alpha Variant) (SARS-CoV-2): ACE2 Inhibitor Screening Colorimetric Assay Kit	78175	96 reactions
Spike Trimer (S1+S2) (B.1.351, Beta Variant) (SARS-CoV-2): ACE2 Inhibitor Screening Colorimetric Assay Kit	78174	96 reactions
Spike Trimer (S1+S2) (B.1.617.2; Delta Variant) (SARS-CoV-2): ACE2 Inhibitor Screening Colorimetric Assay Kit	78275	96 reactions
Spike Trimer (S1+S2) (B.1.617.2.1; Delta Plus Variant) (SARS-CoV-2): ACE2 Inhibitor Screening Colorimetric Assay Kit	78276	96 reactions