Description

The SARS-CoV-2 Spike Trimer (S1+S2):ACE2 [Biotinylated] Inhibitor Screening Colorimetric Assay Kit is designed for screening and profiling inhibitors of the interaction of ACE2 (angiotensin-converting enzyme 2) with the Spike Trimer (S1+S2) protein. This kit comes in a convenient 96-well format, with purified SARS-CoV-2 Spike Trimer (S1+S2) (amino acids 16-1213) and ACE2-Biotin (amino acids 18-740) proteins, streptavidin-HRP, colorimetric HRP substrate, and assay buffer for 100 binding reactions. The SARS-CoV-2 Spike Trimer (S1+S2) protein included in the kit, provides a biologically relevant model for the investigation of SARS-CoV-2/ACE2 interaction.

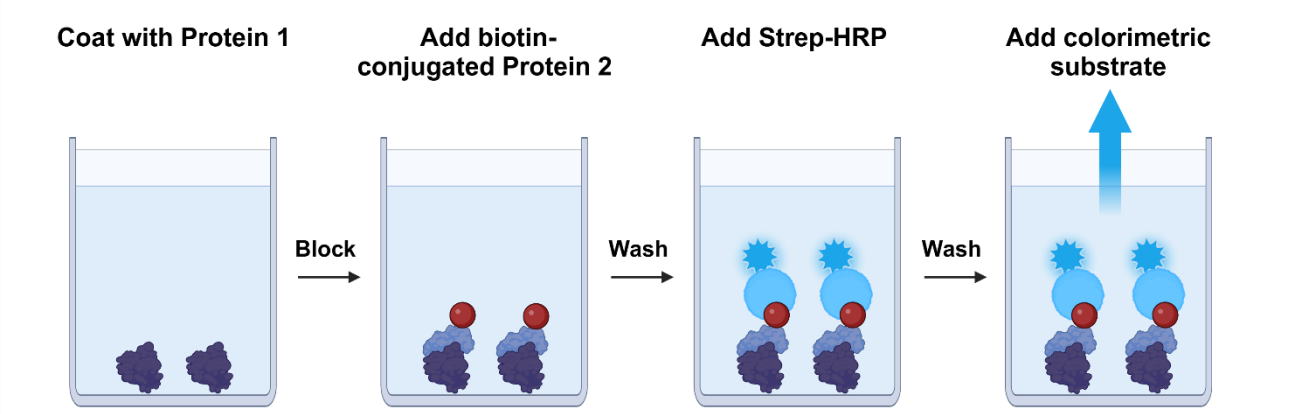

Figure 1. Assay Kit schematic.

First, SARS-CoV-2 Spike Trimer (S1+S2) 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. After a final wash, Colorimetric HRP substrate is added to produce color that can be measured using a UV/Vis spectrophotometer microplate reader. The colorimetric signal is proportional to the efficacy of the binding of Spike Trimer (S1+S2) to ACE2.

Background

The pandemic coronavirus disease 2019 (COVID-19) is caused by Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2). As a first step of the viral replication strategy, the virus attaches to the host cell surface before entering the cell. The Receptor Binding Domain (RBD) of Spike protein 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. It has been widely suggested that active as well as passive immunizations targeting the interaction between the Spike protein of SARS-CoV-2 and ACE2 offer promising protection against the viral infection. However recent reports showed that a mutant strain first identified in the UK (B.1.1.7) exhibits higher transmissibility and infectivity. Investigations on the effects of mutations on viral replication and pathogenesis will be critical for developing effective strategies for vaccines and antibody therapies against COVID-19.

Applications

Screening inhibitors of ACE2 binding to SARS-CoV-2 Spike Trimer (S1+S2).

Supplied Materials

Catalog #	Name	Amount	Storage
100728	Spike Trimer (S1 + S2), His-Tag (SARS-CoV-2)*	5 µg	-80°C
100665	ACE2, His-Avi-Tag, Biotin-Labeled*	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
79964	Transparent 96-well microplate	1	Room Temp

**The initial concentration of the proteins is lot-specific and will be indicated on the tube containing the protein.*

Materials Required but Not Supplied

- 1x PBS (Phosphate-Buffered Saline, pH 7.4)
- 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

This assay kit is compatible with up to 1% final DMSO concentration.

Assay Protocol:

- All samples and controls should be tested in duplicate.
- The assay should include “Blank”, “Positive Control” and “Test Inhibitor” conditions.
- We recommend maintaining the diluted protein on ice during use.
- For detailed information on protein handling please refer to Protein FAQs (bpsbioscience.com).
- 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.
- We recommend Anti- Spike S1 Monoclonal Antibody (SARS-CoV-2) (#100715) as internal control. If not running a dose response curve for the control inhibitor, we recommend running the control inhibitor at 0.1 x, 1 x and 10 x the IC_{50} value shown in the validation data below.
- For instructions on how to prepare reagent dilutions please refer to Serial Dilution Protocol (bpsbioscience.com).

Step 1: Coat 96-well plate

Coat the plate one day prior to running your samples.

1. Thaw **Spike Trimer (S1+S2) protein** on ice. Briefly spin the tube to recover its full content.
2. Dilute **Spike Trimer (S1+S2) protein** to 1 µg/ml in PBS (50 µl/ well).
3. Add 50 µl of diluted **Spike Trimer (S1+S2) protein** solution to each well.
4. Incubate at 4°C overnight.
5. Prepare **1x Assay Buffer** by diluting 3-fold the **3x Immuno Buffer 1** with sterile distilled water.
6. After the overnight coating, discard the solution and wash the plate three times with 100 µl of **1x Assay Buffer**. Tap the plate onto clean paper towels to remove the excess liquid.
7. Block wells by adding 100 µl of **Blocking Buffer 2** to every well.
8. Incubate at Room Temperature (RT) for 1 hour with slow agitation.
9. Remove the blocking solution and wash the plate three times using 100 µl of **1x Assay Buffer** per well.
10. Tap the plate onto clean paper towel to remove the liquid.

Step 2: Binding reaction

1. Prepare the Test Inhibitor/Blocker in **1x Assay Buffer** (25 µl/well): for a titration prepare serial dilutions at concentrations 2-fold higher than the desired final concentrations. The final volume of the reaction is 50 µl.

   1.1. If the Test Inhibitor/Blocker is soluble in water, prepare a solution of the compound that is 2-fold higher than the final desired concentration using **1x Assay Buffer**.

   For the positive and negative controls, use **1x Assay Buffer** (Diluent Solution).

   OR

   1.2 If the Test Inhibitor/Blocker is dissolved in DMSO, prepare a solution of the compound in 100% DMSO that is 100-fold higher than the highest concentration of the serial dilution. Then dilute 50-fold with **1x Assay Buffer** (at this step the compound concentration is 2-fold higher than the desired final concentration). The concentration of DMSO in the dilution is now 2%.

   Prepare serial dilutions of the Test Inhibitor at the desired final concentrations using 2% DMSO in **1x Assay Buffer** to keep the concentration of DMSO constant.

For positive and negative controls, prepare 2% DMSO in **1x Assay Buffer** (vol/vol) so that all wells contain the same amount of DMSO (Diluent Solution).

Note: The final concentration of DMSO should not exceed 1%.

2. Add 25 µl of Test Inhibitor to each well labeled as "Test Inhibitor".
3. Add 25 µl of Diluent Solution to the "Positive Control" and "Blank" wells.
4. Incubate the plate at RT for 30 minutes with slow agitation (neutralizing antibody or protein inhibitor can be preincubated up to 60 minutes).
5. Thaw the **ACE2[B]** on ice. Briefly spin the tube containing the protein to recover its full content.
6. Dilute **ACE2[B]** to 2 ng/µl in **1x Assay Buffer** (25 µl/well).
7. Add 25 µl of diluted **ACE2[B]** to the wells labeled "Test Inhibitor" and "Positive Control".
8. Add 25 µl **1x Assay Buffer** to the wells labeled "Blank".
9. Incubate the plate at RT for 1 hour with slow agitation.

Component	Blank	Positive Control	Test Inhibitor
1x Assay Buffer	25 µl		-
Diluent Solution	25 µl	25 µl	
Test antibody or inhibitor	-	-	25 µl
Diluted ACE2-Biotin (1.5 ng/µl)	-	25 µl	25 µl
Total	**50 µl**	**50 µl**	**50 µl**

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

Step 3: Detection

1. Dilute 1000-fold the **Streptavidin-HRP** using **Blocking Buffer 2** (50 µl/well).
2. Add 50 µl of the diluted **Streptavidin-HRP** to every well.
3. Incubate the plate for 30 minutes at RT with slow agitation.
4. After 30 minutes, discard the solution and wash the plate three times with 100 µl of **1x Assay Buffer**. Tap the plate onto clean paper towels to remove the excess liquid.
5. Add 100 µl of the **Colorimetric HRP Substrate** to each well and incubate the plate at RT until blue color is developed in the "Positive Control" wells. This usually takes ~5 minutes.

Note: The optimal incubation time may vary and should be determined empirically by the user.

6. Prepare enough 1M HCl (aqueous-stop solution) for 100 µl per well to be used.

   Note: Alternatively, 2N H_2SO_4 or other compatible acidic solutions can be used. The optimal incubation time may vary and should be determined empirically by the user.

7. Once a blue color has developed in the "Positive Control" well, add 100 µl of 1M HCl to every well. The blue color should turn yellow.

8. Read the absorbance at 450 nm using a UV/Vis spectrophotometer microplate reader.

9. The "Blank" value should be subtracted from all other values.

Example Results

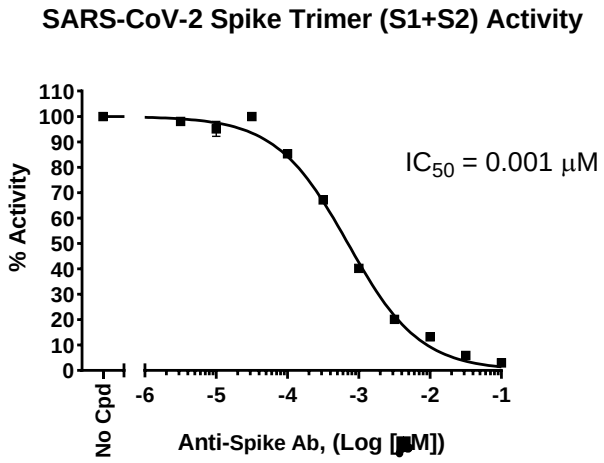

Figure 2. Inhibition of ACE2:SARS-CoV-2 Spike Trimer (S1+S2) binding by an anti-SARS-CoV-2 Spike antibody.
The binding of ACE2 to SARS-CoV-2 Spike Trimer (S1+S2) was measured in the presence of increasing concentrations of an anti-SARS-CoV-2 Spike antibody. Results are expressed as a percentage of binding activity in which the condition without the inhibitor is set to 100%.

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

References

Wang P., *et al.,* 2021 bioRxiv 2021.01.25.428137.
Shen X., *et al.,* 2021 bioRxiv. 2021.01.27.428516.
Hoffman M. *et al.,* 2020 *Cell* 181:1-10.

Troubleshooting Guide

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

Related Products

Products	*Catalog #*	*Size*
ACE2: Spike S1 RBD (SARS-CoV-2) Inhibitor Screening Colorimetric Assay Kit	78031	96 reactions
SARS-CoV-1 Spike Trimer (S1+S2): ACE2 Inhibitor Screening Colorimetric Assay Kit	78012	96 reactions
ACE2, His-Tag Recombinant	11003	20 µg/ 100 µg
ACE2, His-Avi-Tag, Biotin-labeled HiP™ Recombinant	100665	20 µg/ 50 µg
Spike (B.1.1.7 Variant) (SARS-CoV-2) Pseudotyped Lentivirus (Luc Reporter)	78112	100 µl/ 500 µl x 2
Spike (SARS-CoV-2) Pseudotyped Lentivirus (Luciferase Reporter)	79942	100 µl/ 500 µl x 2

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