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Lieferung & Zahlungsart

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Zuschläge

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- Trockeneiszuschlag
- Gefahrgutzuschlag
- Expressversand

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Description

The Spike Trimer (S1+S2) (B.1.617.2; Delta 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 (amino acids 18-740), purified Spike Trimer (S1+S2) (B.1.617.2; Delta Variant) protein (His-tagged) (amino acids 16-1213), 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, Spike Trimer (S1+S2) (Delta 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.

SARS-CoV-2 Variant B.1.617.2, also known as Delta variant, was originally discovered in India and contains deletion Δ156/157 and mutations T19R, G142D, R158G, L452R, T478K, D614G, P681R, D950N.
Drugs targeting the interaction between SARS-CoV-2 Spike protein and human ACE2 may offer some protection against viral infection.

Applications

This kit is useful for screening inhibitors of ACE2 binding to **Spike Trimer (S1+S2) (B.1.617.2; Delta Variant) (SARS-CoV-2).**

Supplied Materials

Catalog #	Name	Amount	Storage
101147	Spike Trimer (S1+S2) (B.1.617.2; Delta Variant), 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 proteins is lot-specific and will be indicated on the tube containing the protein.*

Materials Required but Not Supplied

- 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 diagnostic 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.
- The assay should include "Blank", "Positive Control" and "Test inhibitor".
- 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 maintaining the diluted protein on ice during use.
- For detailed information on protein handling please refer to Protein FAQs (bpsbioscience.com).
- We recommend Spike S1 Neutralizing Antibody (B.1.617.2, B.1.617.2.1, B.1.1.7, B.1.351, B.1.429, and P.1 Variants) (Clone C-A11) (SARS-CoV-2) (#101024) 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).

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

1. Thaw **Spike Trimer (S1+S2) (B.1.617.2; Delta Variant) protein** on ice. Upon first thaw, briefly spin the tube to recover the full contents.

2. Dilute **Spike Trimer protein** to 1 µg/ml in PBS (50 µl/ well).

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

Day 2: Reaction

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

2. After the overnight coating, discard the solution by flipping the plate over a waste container or sink, then tap the plate onto paper towels.

3. Wash the plate three times with 100 µl of **1x Immuno Buffer 1** per well. Tap plate onto clean paper towels to remove liquid.

4. Block by adding 100 µl of **Blocking Buffer 2** to each well.

5. Incubate for 1 hour at Room Temperature (RT) with slow agitation. Remove the blocking solution and tap to dry.

   ****Note there are two methods recommended depending on your type of inhibitor (antibody or small molecule)****

<u>If testing anti-Spike antibody as inhibitor, follow Steps 6-16 below:</u>

6. Prepare dilutions of neutralizing anti-Spike antibody in **1x Immuno Buffer 1** at the desired concentration (it is recommended to use serial dilutions). Prepare enough for 25 µl per well.

7. Add 25 µl of the diluted antibody to the "Test Inhibitor" wells.

8. Add 50 µl of **1x Immuno Buffer 1** to the "Blank" wells.

9. Add 25 µl of **1x Immuno Buffer 1** to the "Positive Control" wells.

10. Incubate the plate for 30 minutes (up to 1 hour) at RT with slow rotation.

11. Thaw the **ACE2-Biotin** on ice. Briefly spin the tube to recover the content of the tube.

12. Dilute ACE2 to 1.5 ng/µl in **1x Immuno Buffer 1** (25 µl/well).

13. After the antibody incubation, add an equal volume (25 µl) of diluted **ACE2-Biotin** to the wells labeled "Test Inhibitor" and "Positive Control".

14. Add 25 µl **1x Immuno Buffer 1** to the wells labeled "Blank". At this step, there should be a total of 50 µl in each well.

15. Incubate the plate at RT for another 1 hour with slow rotation.

Component	Blank	Positive Control	Test Inhibitor
1x Immuno Buffer 1	50 µl	25 µl	-
Test Antibody	-	-	25 µl
	Incubate 30 minutes (up to 1 hour) at RT		
Diluted ACE2-Biotin (1.5 ng/µl)		25 µl	25 µl
Total	**50 µl**	**50 µl**	**50 µl**

16. 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 6-16 below:

6. Add 25 µl of **1x Immuno Buffer 1** to all wells.

7. Prepare the Test Inhibitor (5 µl/well): for a titration prepare serial dilutions at concentrations 10-fold higher than the desired final concentrations. The final volume of the reaction is 50 µl.

   7.1 If the Test Inhibitor is water-soluble, prepare serial dilutions 10-fold more concentrated than the desired final concentrations in distilled water.

   For the positive and negative controls, use distilled water as Diluent Solution.

   OR

   7.2 If the Test inhibitor is soluble in DMSO, prepare the inhibitor in 100% DMSO at a concentration 100-fold higher than the highest desired concentration, then dilute the inhibitor 10-fold in distilled water to prepare the highest concentration of the 10-fold intermediate dilutions. The concentration of DMSO is now 10%.

   Use 10% DMSO in distilled water (vol/vol) for the serial dilution to keep the concentration of DMSO constant.

   For positive and negative controls, prepare 10% DMSO in distilled water (vol/vol) so that all wells contain the same amount of DMSO (Diluent Solution).

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

8. Add 5 µl to each well labeled “Test Inhibitor”.

9. To the “Positive Control” and “Blank” wells, add 5 µl of Diluent Solution.

10. Incubate the plate for 30 minutes (up to 1 hour) at RT with slow rotation.

11. Thaw the **ACE2-Biotin** on ice. Briefly spin the tube to recover the full content of the tube.

12. Dilute ACE-2 in **1x Immuno Buffer 1** to 1.5 ng/µl (20 µl/well).

13. Add 20 µl of **1x Immuno Buffer 1** to the wells labeled "Blank".

14. Add 20 µl of diluted **ACE2-Biotin** to the wells labeled "Test Inhibitor" and "Positive Control".

15. Incubate the plate at RT for 1 hour with slow rotation.

Component	Blank	Positive Control	Test Inhibitor
1x Immuno Buffer 1	45 µl	25 µl	25 µl
Test Inhibitor	-	-	5 µl
Diluent Solution	5 µl	5 µl	-
Incubate 30 minutes (up to 1 hour) at RT			
Diluted ACE2-Biotin (1.5 ng/µl)	-	20 µl	20 µl
Total	**50 µl**	**50 µl**	**50 µl**

16. 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 liquid.

Day 2-Detection:

1. Dilute **Streptavidin-HRP** 1000-fold with **Blocking Buffer 2** (100 µl/well).

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

3. After 30 minutes, discard the solution and wash the plate three times with 100 µl of **1x Immuno Buffer 1.**

4. Block by adding 100 µl of **Blocking Buffer 2** to each well. Incubate for 10 minutes at RT.

5. Remove the blocking solution and tap the plate onto clean paper towels to remove the excess liquid.

6. 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.

7. 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.

8. 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.

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

Example Results

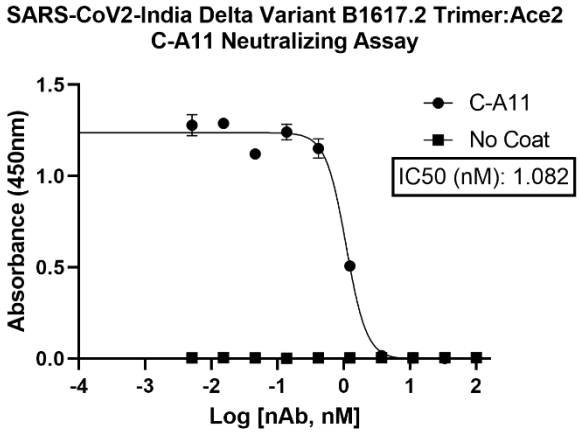

Figure 1: Inhibition of ACE2: Spike Trimer (B.1.617.2; Delta Variant) (SARS-CoV-2) binding by Spike S1 Neutralizing Antibody (B.1.617.2, B.1.617.2.1, B.1.1.7, B.1.351, B.1.429, and P.1 Variants) (Clone C-A11) (SARS-CoV-2).

The binding of ACE2 to Spike Trimer (B.1.617.2; Delta Variant) (SARS-CoV-2) was measured in the presence of increasing concentrations of Spike S1 Neutralizing Antibody (B.1.617.2, B.1.617.2.1, B.1.1.7, B.1.351, B.1.429, and P.1 Variants) (Clone C-A11) (SARS-CoV-2) (#101024). 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*., 2020 Cell; 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

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