

Description

The Spike S1 RBD (B.1.1.7 Variant) (N501Y) (SARS-CoV-2): ACE2 Inhibitor Screening Colorimetric Assay Kit is designed for screening and profiling inhibitors of the interaction of ACE2 with the B.1.1.7 Variant (N501Y) of the SARS-CoV-2 Spike RBD protein. This kit comes in a convenient 96-well format, with purified SARS-CoV-2 Spike S1 RBD B.1.1.7 Variant (N501Y) and ACE2-Biotin proteins, streptavidin-HRP, colorimetric HRP substrate, and assay buffer for 100 binding reactions. The key to this kit is the high sensitivity of detection of ACE2-Biotin protein by Streptavidin-HRP. Only a few simple steps on a microtiter plate are required for the assay. First, Spike S1 RBD protein B.1.1.7 Variant is coated on a 96-well transparent plate. Next, ACE2-Biotin is incubated with Spike S1 RBD B.1.1.7 Variant on the plate. Finally, the plate is treated with streptavidin-HRP followed by addition of an HRP substrate to produce color, which can then be measured using a UV/Vis spectrophotometer microplate reader.

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

This kit is useful for screening for inhibitors of ACE2 binding to SARS-CoV-2 Spike S1 RBD B.1.1.7 Variant (N501Y).

Supplied Materials

Catalog #	Name	Amount	Storage
	Spike S1 RBD (N501Y), His-tag (SARS-CoV-2)*	5 µg	-80°C
100665	ACE2, His-Avi-Tag, Biotin-labeled HiP™*	5 µg	-80°C
79742	Streptavidin-HRP	10 µl	+4°C
79311	3x Immuno Buffer 1	50 ml	-20°C
79728	Blocking Buffer 2	50 ml	+4°C
79651	Colorimetric HRP substrate	10 ml	+4°C
79964	Transparent 96-well microplate	1	Room Temp

*The concentration of Spike S1 and ACE2 is lot-specific and will be indicated on the tubes 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 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.

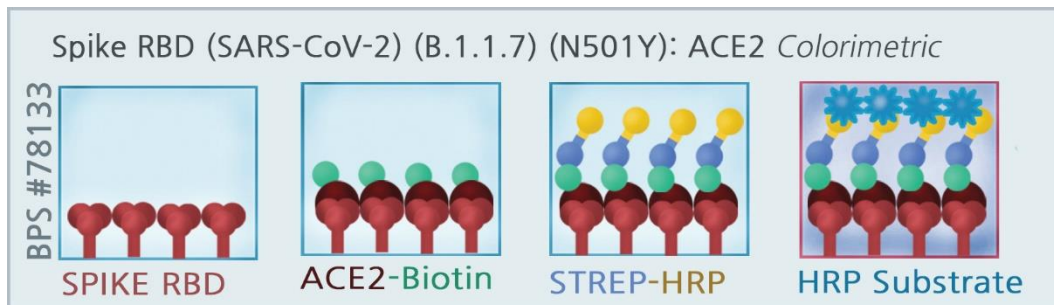
Assay Principle

Figure 1: Assay Principle.

Assay Protocol

- All samples and controls should be tested in duplicate.
- The assay should include “Blank”, “Positive Control” and “Test Sample” conditions.
- We recommend maintaining the diluted protein on ice during use.
- For detailed information on protein handling please refer to [Protein FAQs \(bpsbioscience.com\)](https://www.bpsbioscience.com/protein-faqs).
- We recommend preincubating antibodies or protein inhibitors with the target protein. For small molecule inhibitors, pre-incubation may also be beneficial, depending on the experimental conditions.
- We recommend using ACE2, His-Tag Recombinant (#11003) as internal control. If not running a dose response curve for the control inhibitor, we recommend running the control inhibitor at 0.1X, 1X and 10X the IC₅₀ value shown in the validation data below.
- For instructions on how to prepare reagent dilutions please refer to [Serial Dilution Protocol \(bpsbioscience.com\)](https://www.bpsbioscience.com/serial-dilution-protocol).

Coating the plate with Spike S1 RBD B.1.1.7 Variant (N501Y)

1. Thaw Spike S1 RBD B.1.1.7 Variant (N501Y) on ice. Upon first thaw, briefly spin tube containing Spike S1 RBD B.1.1.7 Variant (N501Y) to recover the full contents of the tube.
2. Dilute Spike S1 RBD to 1 µg/ml with PBS.
3. Add 50 µl of diluted Spike S1 RBD B.1.1.7 Variant (N501Y) solution to each well of the microtiter plate and incubate overnight at 4°C.
4. Dilute 3x Immuno Buffer 1 to 1x Immuno Buffer 1 with distilled water. Dilute only the amount required for the assay; store remaining 3x Immuno Buffer 1 undiluted (~ 60 ml of 1x Immuno Buffer is needed for a 96-well plate).
5. Decant to remove supernatant. Wash the plate three times with 100 µl of 1x Immuno Buffer 1. Tap plate onto clean paper towels to remove liquid.
6. Block wells by adding 100 µl of Blocking Buffer 2 to each well. Incubate for 1 hour at Room Temperature (RT) with slow shaking. Remove supernatant as described in step 5.

Component	Blank	Positive Control	Test Sample
Neutralizing antibody (or Protein inhibitor) in Blocking Buffer 2	-	-	25 µl
Blocking Buffer 2	25 µl	25 µl	-
Biotin-labeled ACE2 (1-2 ng/µl)	-	25 µl	25 µl
Blocking Buffer 2	25 µl	-	-
Total	50 µl	50 µl	50 µl

For the SARS-CoV2 Spike S1 RBD B.1.1.7 Variant (N501Y) neutralizing antibody (or protein inhibitor)

1. Prepare serial dilutions of the test neutralizing antibody or protein inhibitor in Blocking Buffer 2 (25 µl/well).
2. Add 25 µl of the serially diluted neutralizing antibody or protein inhibitor to each well designated "Test Sample." For the wells designated "Blank" and "Positive Control," add 25 µl of Blocking Buffer 2.
3. Incubate the plate for 30 minutes at RT (neutralizing antibody or protein inhibitor can be preincubated up to 60 minutes).
4. Thaw the biotin-labeled ACE2 on ice. After thawing, briefly spin the tube containing biotin-labeled ACE2 to recover the full contents of the tube.
5. Dilute biotin-labeled ACE2 to 1.5 ng/µl with Blocking Buffer 2 (25 µl/well).

6. Add 25 μ l of the diluted biotin-labeled ACE2 to the wells designated "Test Sample" and "Positive Control".
7. For the wells designated "Blank", add 25 μ l Blocking Buffer 2.
8. Incubate the plate for 1 hour at RT with slow agitation.
9. After 1 hour, decant the solution and wash the plate three times with 100 μ l 1x Immuno Buffer 1. Tap plate onto clean paper towels to remove liquid.
10. Dilute Streptavidin-HRP 1000-fold with Blocking Buffer 2.
11. Add 100 μ l to each well. Incubate for 30 minutes at RT with slow agitation.
12. After 30 minutes, decant the solution and wash plate three times with 1x Immuno Buffer 1. Tap plate onto clean paper towel to remove liquid.
13. Add 100 μ l of the Colorimetric HRP substrate to each well and incubate the plate at RT until blue color is developed in the positive control well. This usually takes 1-2 minutes to fully develop. However, the optimal incubation time may vary, and should be determined empirically by the user.
14. After the blue color is developed, add 100 μ l of 1N HCl to each well. Read the absorbance at 450 nm using UV/Vis spectrophotometer microplate reader. The "Blank" wells should exhibit an absorbance of \sim 0.05 at 450 nm. Subtract the "Blank" value from all readings.

Example Results

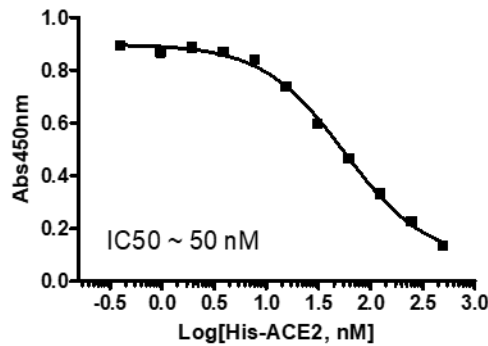


Figure 2: ACE2 protein competes and blocks the binding of biotin-labeled ACE2 to SARS-CoV-2 Spike S1 RBD B.1.1.7 Variant (N501Y).

ACE2, His-Tag Recombinant (#11003) was incubated at increasing concentrations with biotin-labeled ACE2 to SARS-CoV-2 Spike S1 RBD B.1.1.7 Variant (N501Y).

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.

Troubleshooting Guide

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

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-Avi-Tag	11003-1	20 µg
ACE2, His-Avi-Tag, Biotin-labeled HiP™	100665-1	20 µg
Spike (B.1.1.7 Variant) (SARS-CoV-2) Pseudotyped Lentivirus (Luc Reporter)	78112-2	500 µl x 2
Spike (SARS-CoV-2) Pseudotyped Lentivirus (Luc Reporter)	79942-2	500 µl x 2

Version 060324