Spike S1 RBD (B.1.351 Variant) (SARS-CoV-2): ACE2 Inhibitor Screening Colorimetric Assay Kit

#78152
96 reactions

Description

The Spike S1 RBD-B.1.351 (SARS-CoV-2): ACE2 Inhibitor Screening Colorimetric Assay Kit is designed for screening and profiling inhibitors of the interaction of ACE2 with the RBD region of the B.1.351 Variant of the SARS-CoV-2 Spike S1 protein. This kit comes in a convenient 96-well format, with purified Spike RBD-B.1.351 (SARS-CoV-2) 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-B.1.351 is coated on a 96-well transparent plate. Next, ACE2-Biotin is incubated with Spike S1 RBD-B.1.351 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.

A variant strain of SARS-COV-2, identified as B.1.351, was first identified in the fall of 2020 in the Republic of South Africa. This South African variant, also known as 501Y.V2, has many mutations which may lead to higher transmissibility and infectivity. In particular, the B.1.351 variant contains three mutations within the RBD region of the Spike S1 protein: K417N, E484K, and N501Y. Investigating the effect 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 inhibitors of ACE2 binding to Spike S1 RBD-B.1.351 (SARS-CoV-2).

Supplied Materials

Catalog #	Name	Amount	Storage
100978	SARS RBD-B.1.351	5 µg	-80°C
100665	ACE2, His-Avi-Tag, Biotin-labeled HiP™	5 µg	-80°C
79742	Streptavidin-HRP	15 µl	+4°C
79311	3x Immuno Buffer 1	50 ml	-20°C
79728	Blocking Buffer 2	50 ml	+4°C
	Colorimetric HRP substrate	10 ml	+4°C
	Transparent 96-well white microplate	1	Room Temp

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	

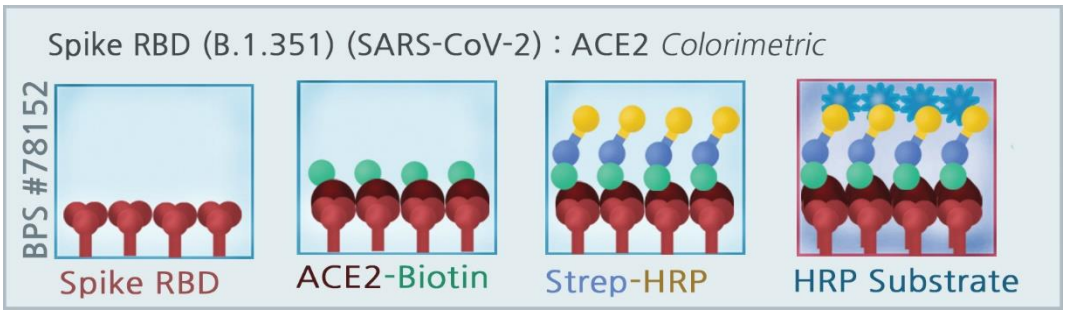
Storage Conditions

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



Assay Protocol

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

Coating the plate with Spike S1 RBD-B.1.351

1. Thaw Spike S1 RBD-B.1.351 on ice. Upon first thaw, briefly spin tube containing Spike S1 RBD-B.1.351 to recover the full contents of the tube. Aliquot into single use aliquots. Immediately store remaining Spike S1 RBD-B.1.351 in aliquots at -80°C. Note: Spike S1 RBD-B.1.351 is very sensitive to freeze/thaw cycles.

Avoid multiple freeze/thaw cycles.

2. Dilute Spike S1 RBD-B.1.351 to 1 µg/ml in PBS.
3. Add 50 µl of diluted Spike S1 RBD-B.1.351 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 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 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 with slow shaking. Remove supernatant as described in step 5.

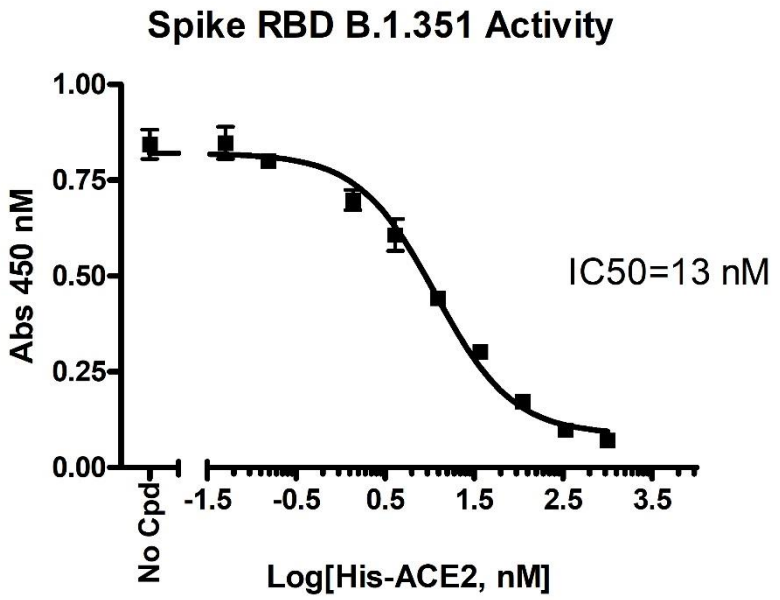
Component	Blank	Positive Control	Test Sample
Neutralizing antibody (or Protein inhibitor) in Blocking Buffer 2	-	-	25 µl
Blocking Buffer 2	25 µl	25 µl	-
Biotin-labeled ACE2 (1-2 ng/µl)	-	25 µl	25 µl
Blocking Buffer 2	25 µl	-	-
Total	50 µl	50 µl	50 µl

For the Spike S1 RBD-B.1.351 neutralizing antibody (or protein inhibitor)

1. Prepare serial dilutions of the test neutralizing antibody or protein inhibitor in Blocking Buffer 2.
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 room temperature (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. Aliquot into single use aliquots. Immediately store remaining biotin-labeled ACE2 in aliquots at -80°C. Note: Biotin labeled ACE2 is very sensitive to freeze/thaw cycles. Avoid multiple freeze/thaw cycles.
5. Dilute biotin-labeled ACE2 to 1.5 ng/µl in Blocking Buffer 2.
6. Add 25 µl of the diluted biotin-labeled ACE2 to the wells designated “Test Sample” and “Positive Control”
7. For the wells designated “Blank,” add 25 µl Blocking Buffer 2.
8. Incubate the plate for 1 hour at room temperature with slow shaking.
9. After 1 hour, decant the solution and wash the plate three times with 100 µl 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 µl diluted Streptavidin-HRP to each well. Incubate for 30 minutes at room temperature with slow shaking.
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 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 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 µl 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 ~ 0.05 at 450 nm. Subtract the "Blank" value from all readings.

Example Results



His-ACE2 protein (BPS Bioscience, #11003) competes with and blocks the binding of ACE2-Biotin to Spike S1 RBD-B.1.351. The experiment was performed using the Spike S1 RBD-B.1.351 (SARS-CoV-2):ACE2 Inhibitor Screening Colorimetric Assay Kit, BPS Bioscience, #78152. Data shown is lot-specific. 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.

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

References

1. Wang P. *et al.,* Increased Resistance of SARS-CoV-2 Variants B.1.351 and B.1.1.7 to Antibody Neutralization. bioRxiv 2021 Jan 26; 2021.01.25.428137
2. Shen X., *et al.,* SARS-CoV-2 variant B.1.1.7 is susceptible to neutralizing antibodies elicited by ancestral Spike vaccines. bioRxiv. 2021 Jan 29; 2021.01.27.428516
3. 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 RBD-B.1.351 (SARS-CoV-2): ACE2 Inhibitor Screening Chemiluminescent Assay Kit	78151	96 reactions
Spike S1 RBD (B.1.351 Variant) Avi-His-Tag (SARS-CoV-2)	100978-1	100 µg
Spike Trimer (S1+S2) (B.1.351 Variant), His-Tag (SARS-CoV-2)	510333	100 µg
Spike S1 RBD (B.1.1.7 Variant) (N501Y) (SARS-CoV-2): ACE2 Inhibitor Screening Colorimetric Assay Kit	78133	96 reactions
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.1.351 Variant) (SARS-CoV-2) Pseudotyped Lentivirus (Luc Reporter)	78142-2	500 µl x 2
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