ACE2: Spike S1 RBD, Mouse Fc-fusion (SARS-CoV-2) Inhibitor Screening Colorimetric Assay Kit

#78031
96 reactions

Description

Coronavirus disease 2019 (COVID-19) increases the risk of developing Acute Respiratory Distress Syndrome (ARDS), which is often fatal at the late stages of infection when the SARS-CoV-2 virus causes significant damage to the lungs. As the first step of the viral replication strategy, the virus attaches to the host cell surface before entering the cell. The Spike protein receptor binding domain (RBD) 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. Drugs targeting the interaction between the Spike protein of SARS-CoV-2 and ACE2 may offer some protection against the viral infection.

The ACE2: Spike S1 RBD, Mouse Fc-fusion (SARS-CoV-2) Inhibitor Screening Colorimetric Assay Kit is designed for screening and profiling inhibitors of this interaction. The key to this kit is the high sensitivity of detection of Fc-tagged Spike S1 protein by HRP-labeled Anti-mouse-Fc. Only a few simple steps on a microtiter plate are required for the assay. First, ACE2 protein is attached to a clear nickel-coated 96-well plate. Next, SARS-CoV-2 Spike S1-Fc is incubated with ACE2 on the plate. Finally, the plate is treated with HRP-labeled anti-Fc, followed by addition of an HRP substrate to produce color, which can then be measured using a UV/Vis spectrophotometer microplate reader.

Applications

This kit is useful for screening or titration of inhibitors of the binding between Spike S1 RBD, Mouse Fc-fusion (SARS-CoV-2) and human ACE2.

Supplied Materials

Catalog #	Name	Amount	Storage
100684	Spike S1 RBD, Mouse Fc-fusion (SARS-CoV-2)*	>1 µg**	-80°C
11003	ACE2, His-Tag*	5 µg	-80°C
52130H	Secondary HRP-labeled antibody 1 (anti-mouse)	10 µl	-80°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
	Nickel-coated 96-well clear microplate	1	+4°C

The initial concentration of both ACE2 and Spike RBD is lot-specific and will be indicated on the tube containing the protein.

****Excess material has been provided for ease of retrieval.**

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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	

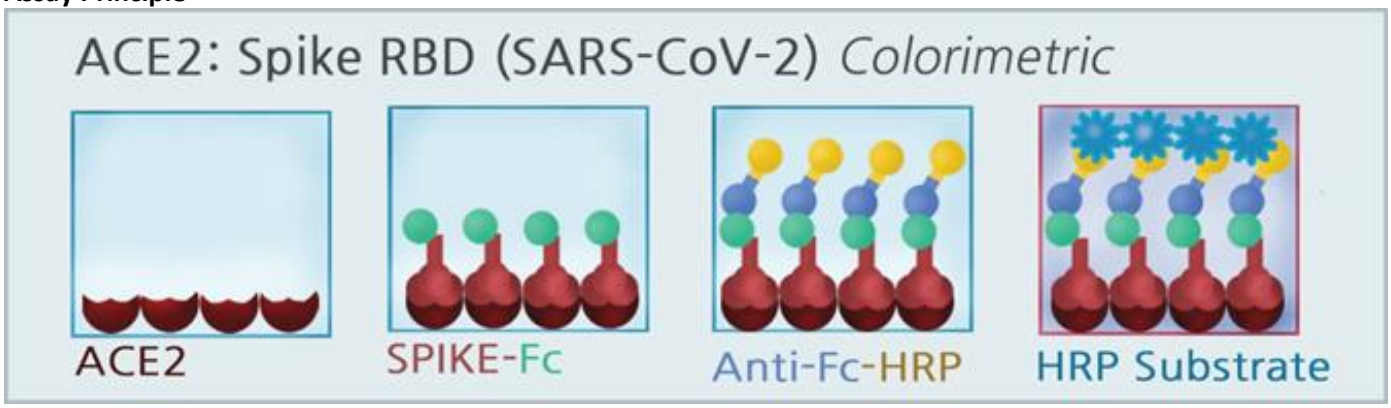
Storage Conditions

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 ACE2, His-Tag

1. Thaw human ACE2, His-Tag on ice. Upon first thaw, briefly spin the tube containing ACE2, His-Tag to recover the full contents of the tube. Dilute ACE2, His-Tag to 1 ng/µl in PBS.
   Note: ACE2, His-Tag is very sensitive to freeze/thaw cycles. Avoid multiple freeze/thaw cycles. Although we do not recommend it, If not using the 96-wells of the assay, dilute only the amount sufficient for the

assay and aliquot the remaining undiluted protein into single use aliquots for future use. Immediately store the remaining material at -80°C.

2. Add 50 µl of diluted ACE2, His-Tag solution to each well of the microtiter plate and incubate for at room temperature for one hour with slow shaking.
3. 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)
4. Wash the plate three times with 100 µl/well of 1x Immuno Buffer 1. After the last wash, tap plate onto clean paper towels to remove leftover liquid.
5. Block by adding 100 µl of Blocking Buffer 2 to each well. Incubate for 10 minutes at room temperature with slow shaking. Wash the plate as described in step 5.

For the neutralizing antibody (or protein inhibitor)

1. Prepare serial dilutions of the test neutralizing antibody or inhibitor compound in Blocking Buffer 2.
2. Add 30 µl of the serially diluted neutralizing antibody or inhibitor compound to each well designated "Test Sample." In the wells designated "Positive Control," add 30 µl of Blocking Buffer 2. In the wells designated "Blank", add 50 µl of Blocking Buffer 2.
3. Incubate the plate for 30 minutes at room temperature (neutralizing antibody or inhibitor can be preincubated for up to 60 minutes).
4. Thaw the Spike S1 RBD, Mouse Fc-fusion (SARS-CoV-2) on ice. After thawing, briefly spin the tube containing the protein to recover the full contents of the tube. Dilute Spike S1 RBD, Mouse Fc-fusion (SARS-CoV-2) to 0.0625 ng/µl in Blocking Buffer 2.
   Note: Spike S1 RBD Mouse Fc-fusion is very sensitive to freeze/thaw cycles. Avoid multiple freeze/thaw cycles. Although we do not recommend it, if not using the 96-wells of the assay, dilute only the amount sufficient for the assay and aliquot the remaining undiluted protein into single use aliquots for future use. Immediately store the remaining material at -80°C.
5.
6. Add 20 µl of the diluted Spike S1 RBD, Mouse Fc-fusion (SARS-CoV-2) to the wells designated "Test Sample" and "Positive Control."

Component	Blank	Positive Control	Test Sample
Neutralizing antibody (or Protein inhibitor) in Blocking Buffer 2	-	-	30 µl
Blocking Buffer 2	50 µl	30 µl	-
Spike S1 RBD, Mouse Fc-fusion (SARS-CoV-2) (0.0625 ng/µl)	-	20 µl	20 µl
Total	50 µl	50 µl	50 µl

7. Incubate the plate for one hour at room temperature with slow shaking.
8. After 1 hour, wash the plate three times with 100 µl 1x Immuno Buffer 1. Tap plate onto clean paper towels to remove liquid.
9. Dilute Secondary HRP-labeled antibody 1 (anti-mouse) 1,000-fold in Blocking Buffer 2.
10. Add 100 µl to each well. Incubate for one hour at room temperature with slow shaking.
11. Wash plate three times with 1x Immuno Buffer 1. Tap plate onto clean paper towel to remove liquid.
12. Add 100 µl of the Colorimetric HRP substrate to each well and incubate the plate at room temperature

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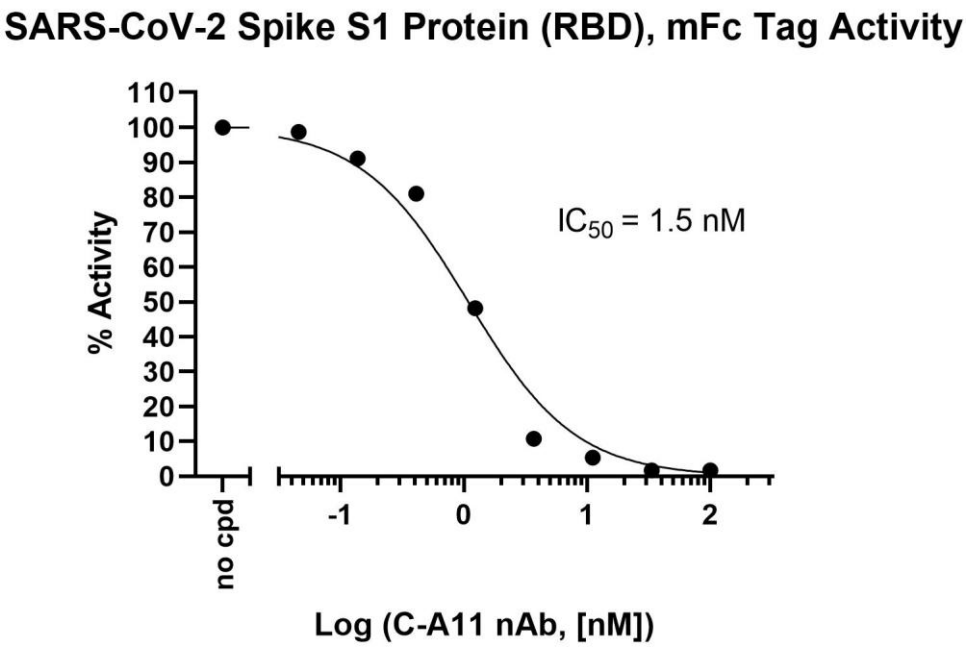
until a blue color has developed in the positive control well. This usually takes 1-2 minutes to fully develop. However, the optimal incubation time may vary, and should be determined empirically by the user.

13. Once the blue color has 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



ACE2: Spike S1 RBD, Mouse Fc-fusion (SARS-CoV-2) Inhibitor Screening Colorimetric Assay Kit, BPS Bioscience, #78031. Inhibition of binding by 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) (BPS Bioscience, #101024) 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

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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, Mouse Fc-fusion (SARS-CoV-2)	100684-1	20 µg
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
ACE2: Spike RBD (SARS-CoV-2) Inhibitor Screening Assay Kit	79936	96 reactions

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