Description

The Spike S1 RBD (B.1.1.7, Alpha Variant) (N501Y) (SARS-CoV-2):ACE2 [Biotinylated] Inhibitor Screening Chemiluminescence Assay Kit is designed for screening and profiling inhibitors or neutralizing antibodies of the interaction between the Spike S1 RBD (B.1.1.7 Variant) (N501Y) protein and human ACE2 (angiotensin-converting enzyme 2). This kit comes in a convenient 96-well format, with Biotinylated-ACE2 (amino acids 18-740), purified SARS-CoV-2 Spike S1 RBD (B.1.1.7 Variant) (N501Y) protein, Streptavidin-HRP, and assay buffers for 100 reactions. The SARS-CoV-2 Spike S1 RBD (B.1.1.7 Variant) (N501Y) protein included in the kit, provides a biologically relevant model for the investigation of SARS-CoV-2/ACE2 interaction.

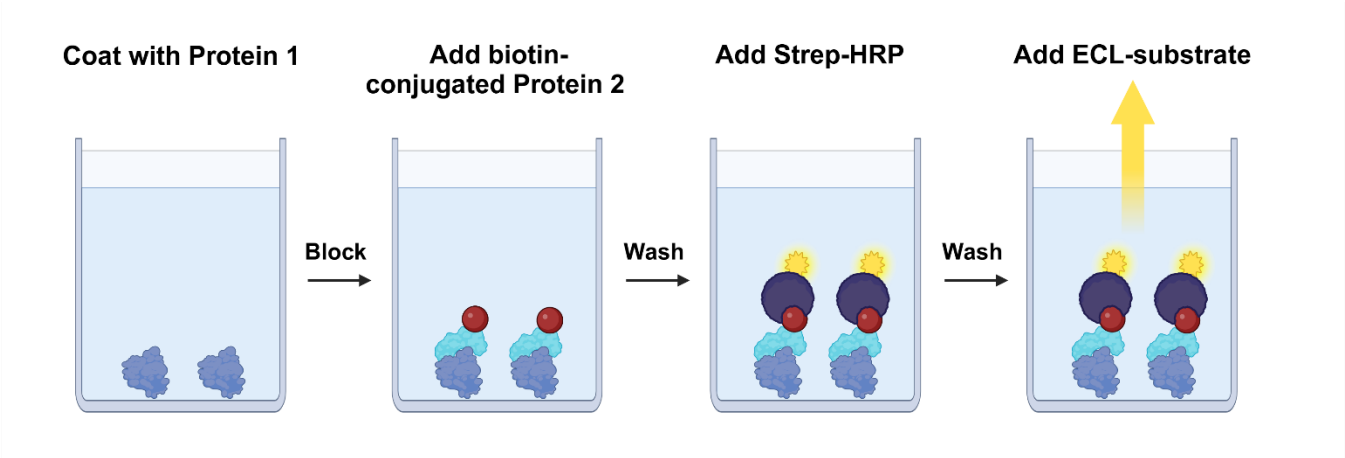

Figure 1. Assay Kit schematic.

First Spike S1 RBD (B.1.1.7 Variant) (N501Y) 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, ELISA ECL substrate is added to produce chemiluminescence that can be measured using a chemiluminescence reader. The chemiluminescence signal is proportional to the efficacy of the binding of Spike S1 RBD (B.1.1.7 Variant) (N501Y) 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. A mutant strain first identified in the UK (B1.1.7) 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 the SARS-CoV-2 Spike S1 (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*	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
79670	ELISA ECL Substrate A (translucent bottle)	6 ml	Room Temp
	ELISA ECL Substrate B (brown bottle)	6 ml	Room Temp
79699	White 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)
- Luminometer or microplate reader capable of reading chemiluminescence
- Adjustable micropipettor and sterile tips
- Rotating or rocker platform

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 performed 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 using ACE2, His-Tag Recombinant (#11003) as internal control. If not running a dose response curve for the control inhibitor/blocker, we recommend running the control inhibitor/blocker at 0.1X, 1X and 10X 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 S1 RBD (B.1.1.7 Variant) (N501Y) protein** on ice. Briefly spin the tube to recover its full content.
2. Dilute **Spike S1 RBD (B.1.1.7 Variant) (N501Y) protein** to 1 µg/ml in PBS (50 µl/well).
3. Add 50 µl of diluted **Spike S1 RBD (B.1.1.7 Variant) (N501Y) 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 tap the plate onto clean paper towel to remove the liquid.

Step 2: Binding reaction

1. Prepare the Test Inhibitor/Blocker in **Blocking Buffer 2** (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 **Blocking Buffer 2**.

   For the positive and negative controls, use **Blocking Buffer 2** (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 **Blocking Buffer 2** (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 **Blocking Buffer 2** to keep the concentration of DMSO constant.

For positive and negative controls, prepare 2% DMSO in **Blocking Buffer 2** (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/Blocker 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 (the 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 1.5 ng/µl in **Blocking Buffer 2** (25 µl/well).
7. Add 25 µl of diluted **ACE2[B]** to the wells labeled "Test Inhibitor" and "Positive Control".
8. Add 25 µl **Blocking Buffer 2** to the wells labeled "Blank".
9. Incubate the plate at RT for 1 hour with slow agitation.

Component	Blank	Positive Control	Test Inhibitor
Blocking Buffer 2	25 µl		-
Diluent Solution	25 µl	25 µl	
Test Inhibitor/Blocker	-	-	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** (100 µl/well).
2. Add 100 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. Just before use, mix 1 volume of ELISA ECL Substrate A and 1 volume of ELISA ECL Substrate B (100 µl of mix/ well).

6. Add 100 µl of mix to every well.

7. Immediately read the plate in a luminometer or microtiter-plate reader capable of reading chemiluminescence.

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

Reading Chemiluminescence:
Chemiluminescence is the emission of light (luminescence) which results from a chemical reaction. The detection of chemiluminescence requires no wavelength selection because the method used is emission photometry and is not emission spectrophotometry.

To properly read chemiluminescence, make sure the plate reader is set for LUMINESCENCE mode. Typical integration time is 1 second, delay after plate movement is 100 msec. Do not use a filter when measuring light emission. Typical settings for the Synergy 2 BioTek plate reader are: use the "hole" position on the filter wheel; Optics position: Top; Read type: endpoint. Sensitivity may be adjusted based on the luminescence of controls.

Example Results

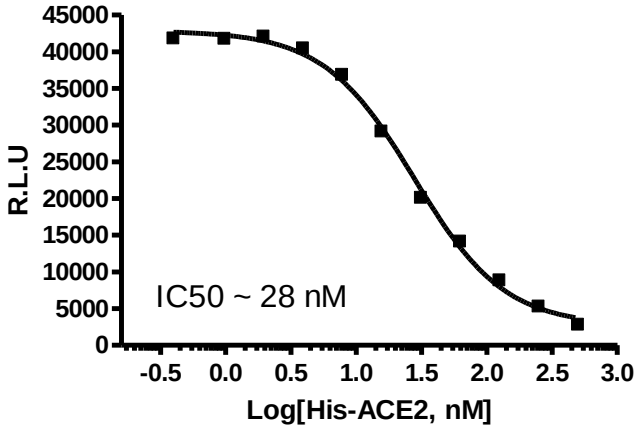

Figure 2. ACE2 protein competes with and blocks the binding of ACE2-Biotin to Spike S1 RBD B.1.1.7 Variant (N501Y).

ACE2, His-Tag Recombinant protein (#11003) competes with and blocks the binding of biotin-labeled ACE2 to SARS-CoV-2 Spike S1 RBD B.1.1.7 Variant (N501Y). Results are expressed as a percentage of binding activity in which the condition without the neutralizing antibody is set to 100%.

Data shown is representative. For lot-specific information, please contact BPS Bioscience, Inc. 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*
Spike S1 RBD (B.1.1.7 Variant) (N501Y) (SARS-CoV-2): ACE2 Inhibitor Screening Colorimetric Assay Kit	78133	96 reactions
Spike S1 RBD (SARS-CoV-2): ACE2 Inhibitor Screening Assay Kit	79931	96 reactions
ACE2 : Spike S1 RBD (SARS-CoV-2) Inhibitor Screening Assay Kit	79936	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
Spike (B.1.1.7 Variant) (SARS-CoV-2) Pseudotyped Lentivirus (Luc Reporter)	78112-2	500 µl x 2

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