FOR RESEARCH USE ONLY!

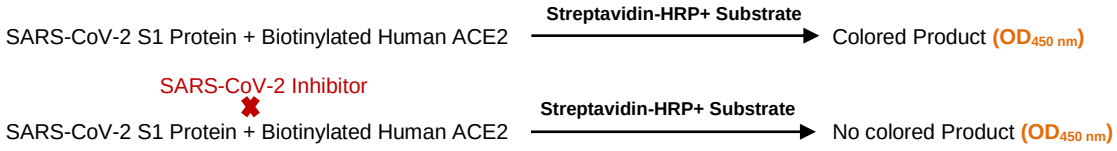
SARS-CoV-2 S1 Protein-ACE2 Binding Inhibitor Screening Kit

07/20

(Catalog # K2050-100; 100 assays; Store at -20°C)

I. Introduction:

Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2), also known as the 2019 Novel Coronavirus (2019-nCoV) or human coronavirus 2019 (HCoV-19 or hCoV-19), is the cause of the Coronavirus Disease 2019 (COVID-19) pandemic. It is a RNA virus that causes severe respiratory diseases in humans. SARS-CoV-2 coronavirus contains four main structural proteins namely Spike (S), Membrane (M), Envelope (E), and Nucleocapsid (N) protein. Spike protein is located on the outer envelope of the virion and mediates the viral entry and thus, plays an important role in inducing neutralizing antibodies and protective immunity. S protein consists of S1 and S2 subunits. The S1 subunit contains a receptor-binding domain that can specifically bind to the host-receptor namely Angiotensin Converting Enzyme 2 (ACE2), which facilitates the entry of the virus into the target cells including respiratory or intestinal epithelial cells, endothelial cells, alveolar monocytes or macrophages. The receptor recognition step is an important determinant of the viral infectivity, pathogenesis & host range. Therefore, an intervention strategy that targets S1 protein and ACE2 interaction presents an important target for vaccination or antiviral strategies that includes small molecules and therapeutic antibodies. **BioVision's SARS-CoV-2 S1 Protein-ACE2 Binding Inhibitor Screening Kit** can be used to screen for potential inhibitors of S1 protein binding to human ACE2. In this assay, the binding of S1 protein to biotinylated human ACE2 is detected using Streptavidin-HRP. Subsequently, a TMB substrate is added to visualize the HRP enzymatic reaction thereby generating a blue colored product that changes to yellow once the stop solution is added. The density of the yellow color is proportional to the binding of S1 protein to Human ACE2. However, in the presence of potent inhibitor(s), the binding of S1 protein to Human ACE2 is suppressed thereby preventing the color generation. The assay kit is adapted to a 96-well format and provides a reliable test for high throughput screening of potential inhibitors of S1 protein binding to Human ACE2.



II. Application:

- Screening or characterizing inhibitors of SARS-CoV-2, S1 protein binding to human ACE2

III. Kit Contents:

Components	K2050-100	Cap Code	Part Number
S1 Protein coated Microplate	8 x 12 strips	---	K2050-100-1
Biotinylated Human ACE2	1 vial	Green	K2050-100-2
Streptavidin-HRP	25 µl	Amber	K2050-100-3
SARS-CoV-2 Inhibitor	70 µl	Red	K2050-100-4
TMB Substrate	20 ml	Amber/NM	K2050-100-5
Stop Solution	20 ml	NM	K2050-100-6
Wash Buffer (10X)	50 ml	NM	K2050-100-7
Assay Diluent	50 ml	Blue	K2050-100-8
Plate Sealers	4	---	K2050-100-9

IV. User Supplied Reagents and Equipment:

- Microplate reader capable of measuring absorbance at 650 nm and 450 nm
- Adjustable pipettes and pipette tips. Multichannel pipettes are recommended
- Deionized water
- Eppendorf tubes
- Absorbent paper

V. Storage Conditions and Reagent Preparation:

Store the kit at -20°C, protected from light. Briefly centrifuge small vials prior to opening. Read the entire protocol before performing the assay. Upon opening, use within two months.

- **S1 Protein coated Microplate:** Store at -20°C
- **Biotinylated Human ACE2:** Reconstitute the vial in 60 µl Assay Diluent. Divide into aliquots & store at -20°C. Keep on ice while in use.
- **Streptavidin-HRP:** After opening, store at 4°C, protected from light.
- **TMB Substrate, Stop Solution and Assay Diluent:** After opening, **s**tore at 4°C. Bring to room temperature (RT) before use.
- **SARS-CoV-2 Inhibitor:** Divide into aliquots and store at -20°C. Keep on ice while in use.
- **Wash Buffer (10X):** Bring the bottle to RT. Prepare 1X Wash Buffer for the assay by diluting the 10X Wash Buffer with dH_2O. The 1X Wash Buffer can be stored at 4°C for one month.

VI. SARS-CoV-2 S1 Protein and Human ACE2 Binding Inhibitor Screening Protocol:

1. Screening Compounds, Inhibitor Control and Background Control Preparation:

Note: Wash the S1 Protein coated Microplate strip(s) one time with 1X Wash Buffer (250 µl/well) before adding the reagents.
Sample compound [S]: Dissolve the sample compound(s) at 50X or higher concentration in an appropriate solvent. Further dilute to 2X (the desired concentration) with Assay Diluent. Candidate antibodies can be prepared to proper dilution with Assay Diluent. Human serum can be diluted to at least 1:50 fold with Assay Diluent. Add 50 µl of diluted sample compound(s) or candidate samples into the designated wells of the S1 Protein pre-coated microplate(s).
Binding Control [Binding] (No Inhibitor): Add 50 µl of Assay Diluent to the designated well(s).

Background Control [BC]: Add 100 µl of Assay Diluent to the designated well(s).
Inhibitor Control [IC]: Prepare 10-fold dilution of the SARS-CoV-2 Inhibitor by adding 6 µl of the SARS-CoV-2 Inhibitor to 54 µl Assay Diluent. Mix well and add 50 µl of diluted SARS-CoV-2 Inhibitor solution into the designated well(s).
Incubate the plate by shaking gently at RT for 30 min, protected from light.

	[S]	[IC]	[Binding]	[BC]
Diluted Sample Compound(s)	50 µl	-	-	-
Diluted SARS-CoV-2 Inhibitor	-	50 µl	-	-
Assay Diluent	-	-	50 µl	100 µl

Note: Additional wells with serial dilutions of the sample compound(s), candidate antibodies or human serum may be prepared at this time, if desired. Each well should contain 50 µl samples at the desired concentration.

2. **Diluted Biotinylated Human ACE2 Preparation and Addition:** Prepare 100 fold dilution of the Biotinylated Human ACE2 by mixing 2 µl of reconstituted Biotinylated Human ACE2 with 198 µl of Assay Diluent and mix well. Add 50 µl of **diluted Biotinylated Human ACE2** to each well containing Sample **[S]**, Inhibitor Control **[IC]**, and Binding Control **[Binding]**. Cover the plate with a plate sealer. **Incubate the plate by shaking gently at RT for 2 hr, protected from light.**

   Notes:

   a). Discard unused, diluted Biotinylated Human ACE2 Solution.

   b). Do not add the diluted Biotinylated Human ACE2 to [BC] wells.

3. Aspirate all the reagents and wash each well 3 times. Wash by filling each well with 250 µl of 1X Wash Buffer and incubating for 3-5 min. Remove the 1X Wash Buffer completely before the next wash. After the last wash, remove any remaining Wash Buffer by aspiration or decanting. Clap the plate on absorbent filter papers or other absorbent materials.

   Note: Complete removal of Wash Buffer is essential for accurate results.

4. **Diluted Streptavidin-HRP Preparation:** Prepare 1:20 dilution of Streptavidin-HRP by adding 2 µl of stock streptavidin-HRP to 38 µl Assay Diluent. Further prepare 1:100 dilution of Streptavidin-HRP by adding 40 µl of diluted Streptavidin-HRP to 3960 µl Assay Diluent and mix well. Add 100 µl of diluted Streptavidin-HRP into each well and **incubate the plate by shaking gently at RT for 1 hr, protected from light**. After incubation, remove the solution and wash wells with 250 µl 1X Wash Buffer (4X, 3-5 min each). After the last wash, remove any remaining Wash Buffer by aspiration or decanting. Clap the plate on absorbent filter papers or other absorbent materials.

   Note: Complete removal of Wash Buffer is essential for accurate results.

5. **Measurement:** Add 100 µl TMB Substrate into all wells & measure the absorbance at 650 nm for 2-20 min at RT and monitor the color development. Add 100 µl Stop Solution into each well to stop the reaction & immediately measure the absorbance at 450 nm.

   Notes:

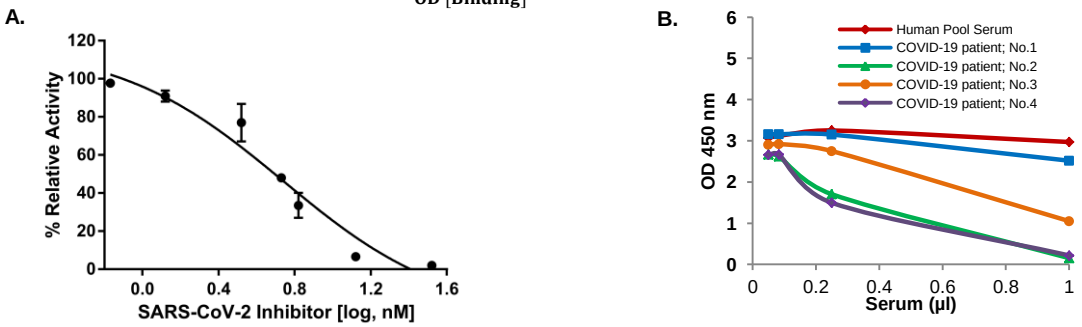
   a). Incubation time following addition of the TMB substrate must be optimized to avoid over development of the color. Recommended absorbance (OD 650 nm) for Binding Control well is 0.8-1. After adding the Stop Solution, mix well and read the plate immediately.

   b). The OD value at 450 nm will be roughly twice the OD value at 650 nm.

6. **Calculation:** Calculate the $OD_{450\ nm}$ for all wells including [S], [Binding], and [BC]. Subtract the $OD_{450\ nm}$ of [BC] well from the $OD_{450\ nm}$ of [S] and [Binding] wells.

$$\% \text{ Relative Inhibition} = \frac{OD[\text{Binding}] - OD[S]}{OD[\text{Binding}]} \times 100$$

$$\% \text{ Relative Activity} = \frac{OD[S]}{OD[\text{Binding}]} \times 100$$



Figures: (a). Inhibition of S1 Protein and Human ACE2 binding by SARS-CoV-2 Inhibitor. (b). Inhibition of S1 protein and Human ACE2 binding by COVID-19 patients' serum(s). COVID-19 was confirmed in patients using Roche Swab. Assay was carried out following the kit protocol.

VII. Related Products:

ACE2 Inhibitor Screening Kit (K310)
SARS-CoV-2 Nucleoprotein IgG Antibody ELISA Kit (E4821)
Anti-SARS-CoV-2 Spike S1 Antibody (A3000)
Anti-CoV-2&SARS-CoV S1 Antibody (A2103)
ACE2, Human Recombinant (P1535)
Angiotensin II Converting Enzyme (ACE2) Activity Kit (K897)
Coronavirus Rapid RT-qPCR Detection Kit (K1461)
Human CellExp™ Coronavirus Spike Protein ((P1547)
Recombinant SARS-CoV-2 Spike Protein S1 (His-tag) (P1540)
Recombinant SARS-CoV-2 Spike Protein S1 (Fc tag) (P1541)

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