Technical Bulletin

SARS-CoV-2 Inhibitors Screening Kit

CS2000

Product Description

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) belongs to *Coronaviridae* (CoVs), a large family of viruses that usually cause mild to moderate upper-respiratory tract diseases.[1] CoVs are single-stranded RNA viruses that have a high mutation rate, and are therefore highly diverse.[1] Their hosts include human and non-human mammals, as well as birds.[1]

SARS-CoV-2 infects cells which express angiotensin converting enzyme 2 (ACE2).[2] ACE2 binds to the viral envelope-associated spike (S) protein as a cellular receptor, following proteolytic cleavage of both S and ACE2 by serine proteases.[3] S protein is believed to be the target of neutralizing antibodies, because it is the main trans-membrane glycoprotein responsible for receptor-binding and virion entry.[4]

The S protein is a trimeric protein. During the binding to ACE2, S protein undergoes a substantial structural rearrangement to facilitate the fusion of the viral membrane with the host cell membrane.[5] The receptor binding domain (RBD) of S protein is the domain that specifically binds to ACE2. RBD is the major target for neutralizing antibodies for coronaviruses[6], and anti-RBD antibodies likely correlate with virus neutralization.[7]

The SARS-CoV-2 Inhibitors Screening Kit is a convenient tool to screen for inhibitors of the ACE2:RBD protein-protein interaction. The kit is based on a colorimetric ELISA, where biotinylated ACE2 (Biotin-ACE2) and Extravidin®-peroxidase are used to detect the ACE2:RBD interaction. One molecule of Extravidin® binds four molecules of biotin by a non-covalent interaction that is essentially irreversible. Binding visualization is achieved by using *o*-Phenylenediamine dihydrochloride (OPD), a peroxidase substrate with absorbance at 450 nm. Inhibitors and neutralizing antibodies against RBD that interfere with the ACE2:RBD interaction will result in reduced colorimetric signal.

Components

This kit contains sufficient reagents for 200 assays in 96-well plates.

Component	Component Number	Amount	Cap Color
RBD	CS2000A	50 µg	Red cap
Biotin-ACE2	CS2000B	25 µg	Green cap
ExtrAvidin®-Peroxidase	CS2000C	30 µL	Yellow cap
SIGMA*FAST*™ OPD	P9187	5 tablet set	N/A

Components Information

- RBD: 50 µg of lyophilized RBD. Reconstitute with 500 µL of water to a final concentration of 100 µg/mL. To avoid freeze/thaw cycles, it is recommended to prepare aliquots, and store the aliquots at –20 °C.
- Biotin-ACE2: 25 µg of lyophilized Biotin-ACE2. Reconstitute with 250 µL of water to 100 µg/mL. Avoid freeze/thaw cycles. Store at -20 °C.
- ExtrAvidin®-Peroxidase: Ready-to-use, store at 2-8 °C. **Do Not Freeze**.
- SIGMA*FAST*™ OPD: Each SIGMA*FAST*™ OPD tablet set consists of one OPD tablet (silver foil) and one urea hydrogen peroxide tablet (gold foil). Store the tablet sets at 2-8 °C. Each tablet set, when dissolved in 20 mL of water, provides 20 mL of ready-to-use substrate solution. The substrate solution should be used within one hour. Protect from light.

Equipment Required But Not Provided

- 96-well microtiter flat-bottom plates.

  Note: It is extremely important to use a plate with high protein-binding capacity. The use of Cat. No. M9410 is recommended.

- 0.01 M Phosphate Buffered Saline (PBS), pH 7.2-7.4 (such as Cat. No. P3813).
- PBS-T, Phosphate Buffered Saline with 0.05% TWEEN® 20, pH 7.2-7.4, as the Wash Buffer (such as Cat. No. P3563).
- 1% BSA in PBS (such as Cat. No. P3688).
- Ultrapure water, for reconstitution of components RBD and Biotin-ACE2.
- Plate reader equipped with a 450 nm filter **Optional**: with a 492 nm filter for end point reading (upon addition of 3M HCl or 3M H_2SO_4)
- 37 °C incubator.
- **Optional:** Microtiter Plate Washer
- **Optional:** 3 M HCl or 3 M H_2SO_4 stop solution

Storage/Stability

Upon receipt, store all components at 2-8 °C. The unopened kit is stable for 2 years as supplied.

Precautions and Disclaimer

For Research Use Only. Not for use in Diagnostic Procedures. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Sample Preparation Instructions

Prepare a sample inhibitor solution by diluting sample inhibitors (for example, antiserum) with PBS to the final testing concentration. An initial concentrated inhibitor solution may be in a different solvent if the inhibitor is minimally soluble in aqueous PBS. For unknown samples, it is suggested to test several sample dilutions.

Serum/plasma preparation: It is suggested to dilute the serum/plasma 1:10 with PBS. This initial dilution should be further diluted 2-fold (for example, 1:20, 1:40, 1:80 etc.).

Procedure

The assay is formatted for a 96-well microplate. All samples and controls should be run in duplicate. Equilibrate all reagents to room temperature before use. Briefly centrifuge vials before opening. For unknown samples, it is suggested to test several dilutions to ensure a dose-response effect.

1. **Coating**

   Prepare a 2 µg/mL RBD coating solution: For each well, dilute 2 µL of the 100 µg/mL RBD with 98 µL of PBS (1:50). Pipette 100 µL/well of this coating solution to the appropriate wells of the microtiter plate. Cover the plate and incubate for 1 hour at 37 °C or overnight at 4 °C.

2. **Washing**

   Aspirate the contents of each well and wash with 200 µL/well of PBS-T. Aspirate and discard the PBS-T. Repeat this process three times. Upon the third wash, aspirate the contents of each well, leaving the wells dry. To obtain consistent results, the use of a microtiter plate washer is recommended.

3. **Blocking**

   To each well of the microtiter plate, add 150 µL/well of 1% BSA in PBS. Incubate for 30 minutes at 37 °C.

4. **Washing**

   Aspirate the contents of each well and wash with 200 µL/well of PBS-T. Aspirate the contents of each well, leaving the wells dry. To obtain consistent results, the use of a microtiter plate washer is recommended.

5. **Sample**

   Dilute the tested inhibitor (such as serum) to the desired dilution with PBS (see Sample Preparation Instructions section). A starting dilution of 1:10 is recommended. As a negative control, use naïve serum (for example, serum from noninfected individuals). Always include a positive control, where no inhibitor is added. A final volume of 50 µL/well is required for each sample. Add 50 µL of sample to each sample well and incubate for 60 minutes at 37 °C.

CS2000bul Rev 10/21 BR,LN,NA,ER,LB,GCY

Summary of the Assay Setup: Reaction Volumes

	Sample	Positive control	Blank
Serum or inhibitor	50 µL	-	-
PBS	-	50 µL	100 µL
Biotin-ACE2	50 µL	50 µL	-

6. **Dilute** the 100 µg/mL Biotin-ACE2 working solution 200-fold in PBS for a final concentration of 0.5 µg/mL. For 20 wells, 5 µL of the 100 µg/mL Biotin-ACE2 solution should be diluted with 995 µL of PBS. Add 50 µL of the diluted Biotin-ACE2 to each well (including the positive control wells). As a blank, allocate wells where no Biotin-ACE2 is added. Incubate for 60 minutes at 37 °C.
7. **Washing**

   Aspirate the contents of each well and wash with 200 µL/well of PBS-T. Aspirate and discard the PBS-T. Repeat this process three times. Upon the third wash, aspirate the contents of each well, leaving the wells dry. To obtain consistent results, the use of a microtiter plate washer is recommended.
8. **Dilute** 5 µL of ExtrAvidin®-Peroxidase with 10 mL of PBS-T. Add 100 µL of the diluted ExtrAvidin®-Peroxidase and incubate for 40 minutes at 37 °C.
   While in incubation, prepare the SIGMA*FAST*™ OPD solution: Dissolve one OPD tablet (silver foil) and one urea hydrogen peroxide tablet (gold foil) in 20 mL purified water. Mix by vortexing until fully dissolved.
   Note: the substrate solution should be used within one hour. Protect from light.
9. **Washing**

   Aspirate the contents of each well and wash with 200 µL/well of PBS-T. Aspirate and discard the PBS-T. Repeat this process three times. Upon the third wash, aspirate the contents of each well, leaving the wells dry. To obtain consistent results, the use of a microtiter plate washer is recommended.
10. **Add** 100 µL of the SIGMA*FAST*™ OPD solution to each sample, positive control and blank wells. Incubate the plate, protected from light, for 5-10 minutes at room temperature. During the incubation, inspect the plate to see if color develops. Measure the absorbance at 450 nm. **Optional:** Add 25 µL of 3 M HCl or 3 M H_2SO_4 to stop the reaction and measure the absorbance at 492 nm.

Note: For convenience, an Excel-based calculation sheet is available on the CS2000 Product Detail Page. Use this sheet to calculate the amounts of reagents required, as well as to calculate the test results.

Results

Calculations

- An Excel-based calculation sheet is available at the Product Detail Page. Use this sheet to calculate the test results.
- If the Excel-based calculation sheet at the Product Detail Page is not used, calculations should be performed as follows:

To calculate the inhibition rate, use the following equation:

$$\% \text{ inhibition} = \frac{(P.C. - B) - (S - B)}{(P.C. - B)} \times 100$$

Where:

P.C. = Positive control (no inhibitor)

B = Blank (no Biotin-ACE2)

S = Sample (inhibitor/serum or negative control, such as the naïve serum)

CS2000bul Rev 10/21 BR,LN,NA,ER,LB,GCY

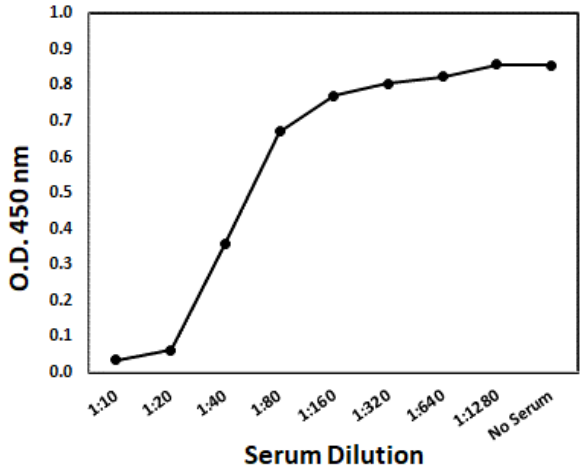

Figure 1. Inhibition of RBD:ACE2 interaction by serum from patients vaccinated with the Pfizer-BioNTech COVID-19 vaccine.

Serum was diluted as indicated, and was used to assess the ability to neutralize the RBD:ACE2 interaction, according to the kit's protocol.

References

1. Hamed, M.A., *Bull. Natl. Res. Cent.*, **44(1)**, 86 (2020).
2. St. John, A.L., and Rathore, A.P.S., *J. Immunol.*, **205(3)**, 555-564 (2020).
3. Heurich, A.H., *J. Virol*., **88(2)**, 1293-1307 (2014).
4. Wrapp, D. *et al.*, *Science*, **367(6483)**, 1260-1263 (2020).
5. Li, F., *Annu. Rev. Virol.*, **3(1)**, 237-261 (2016).
6. Berry, J.D. *et al.*, *MAbs*, **2(1)**, 53-66 (2010).
7. Amanat, F. *et al.*, *Nat. Med.*, **26(7)**, 1033-1036 (2020).

Notice

We provide information and advice to our customers on application technologies and regulatory matters to the best of our knowledge and ability, but without obligation or liability. Existing laws and regulations are to be observed in all cases by our customers. This also applies in respect to any rights of third parties. Our information and advice do not relieve our customers of their own responsibility for checking the suitability of our products for the envisaged purpose.

The information in this document is subject to change without notice and should not be construed as a commitment by the manufacturing or selling entity, or an affiliate. We assume no responsibility for any errors that may appear in this document.

Technical Assistance

Visit the tech service page at SigmaAldrich.com/techservice.

Standard Warranty

The applicable warranty for the products listed in this publication may be found at SigmaAldrich.com/terms.

Contact Information

For the location of the office nearest you, go to SigmaAldrich.com/offices.

CS2000bul Rev 10/21 BR,LN,NA,ER,LB,GCY