

SARS-CoV-2 (B.1.351) Inhibitor Screening Kit (Spike RBD)

Pack Size: 96 tests

Catalog Number: EP-109

IMPORTANT: Please carefully read this manual before performing your experiment.

For Research Use Only. Not For Use In Diagnostic Or Therapeutic Applications

INTENDED USE

This kit is developed for screening inhibitors of SARS-CoV-2(B.1.351).

It is intended for research use only (RUO).

PRINCIPLE OF THE ASSAY

Multiple variants of SARS-CoV-2 are circulating globally and posing new challenges to human health. As concerns over the potential impacts of the variants grow, the development of efficient treatment against multiple variants of SARS-CoV-2 is an emergent need for global public health. It is necessary to develop SARS-CoV-2 inhibitors, such as vaccines, therapeutic antibodies and small molecule compounds against SARS-CoV-2(B.1.351). Therefore, it is helpful to develop the SARS-CoV-2 (B.1.351) Inhibitor Screening Kit (Spike RBD) to test SARS-CoV-2 inhibitors.

This inhibitor screening ELISA kit is designed to facilitate the identification and characterization of SARS-CoV-2(B.1.351) inhibitors. The microplate in the kit has been pre-coated with Human ACE2 protein. First samples are added to the wells followed by addition of HRP-SARS-CoV-2 Spike RBD(K417T, E484K, N501Y). After incubation, the wells are washed and substrate is added to the wells. The reaction is terminated by the addition of stop solution and the intensity of absorbance is measured at 450 nm.

MATERIALS PROVIDED

TABLE 1. MATERIALS PROVIDED

Catalog	Components	Size (96 tests)	Format	Storage	
				Unopened	Opened
EP109-C01	Pre-coated Human ACE2 Microplate	1 plate	Solid	2-8°C	2-8°C
EP109-C02	SARS-CoV-2 Inhibitor	10 µg	Powder	2-8°C	-70°C
EP109-C03	HRP-SARS-CoV-2 Spike RBD(B.1.351)	15 µg	Powder	2-8°C, avoid light	-70°C, avoid light
EP109-C04	10xWashing Buffer	50 mL	Liquid	2-8°C	2-8°C
EP109-C05	Dilution Buffer	50 mL	Liquid	2-8°C	2-8°C
EP109-C06	Substrate Solution	12 mL	Liquid	2-8°C, avoid light	2-8°C, avoid light
EP109-C07	Stop Solution	7 mL	Liquid	2-8°C	2-8°C

STORAGE AND VALIDITY INSTRUCTIONS

The unopened kit is stable for 12 months from the date of manufacture if stored at 2°C to 8°C.

The opened kit should be stored per TABLE 1. The shelf life is 30 days from the date of opening.

Note:

- a. Do not use reagents past their expiration date.
- b. Find the expiration date on the outside packaging.

MATERIALS REQUIRED BUT NOT PROVIDED

Single or dual wavelength microplate reader with 450 nm filter;

Centrifuge;

37 °C Incubator;

Single channel or multichannel pipettes with 10 µL, 200 µL and 1000 µL precision;

10 µL, 200 µL and 1000 µL pipette tips;

Test Tubes;

Graduated cylinder;

Deionized or distilled water for dilution;

REAGENT PREPARATION

1. Bring all reagents and samples to preparation temperature (20°C-25°C) before use.

2. As recommended in Table 2, the lyophilized materials of SARS-CoV-2 Inhibitor and HRP-SARS-COV-2 Spike RBD(B.1.351) will be diluted into a rehydrated solution with ultrapure water/deionized water. Before use, the rehydrated solution needs to be balanced at room temperature of 30 min, shake gently every 10 min. Do not shake or vortex violently. The rehydrated solution should be stored at -70°C, Do not thaw and freeze more than 3 times.

TABLE 2. RECONSTITUTION METHODS FOR 96 TESTS

Catalog	Components	Amount	Stock Solution Con.	Reconstitution Buffer and Vol.
EP109-C02	SARS-CoV-2 Inhibitor	10 µg	100 µg/mL	100µL
EP109-C03	HRP-SARS-CoV-2 Spike RBD(B.1.351)	15 µg	100 µg/mL	150µL

RECOMMENDED SAMPLE PREPARATION

1. Working fluid preparation

1.1 Preparation of 1×Washing Buffer:

Dilute 50 mL 10×Washing Buffer with ultrapure water/deionized water to 500 mL.

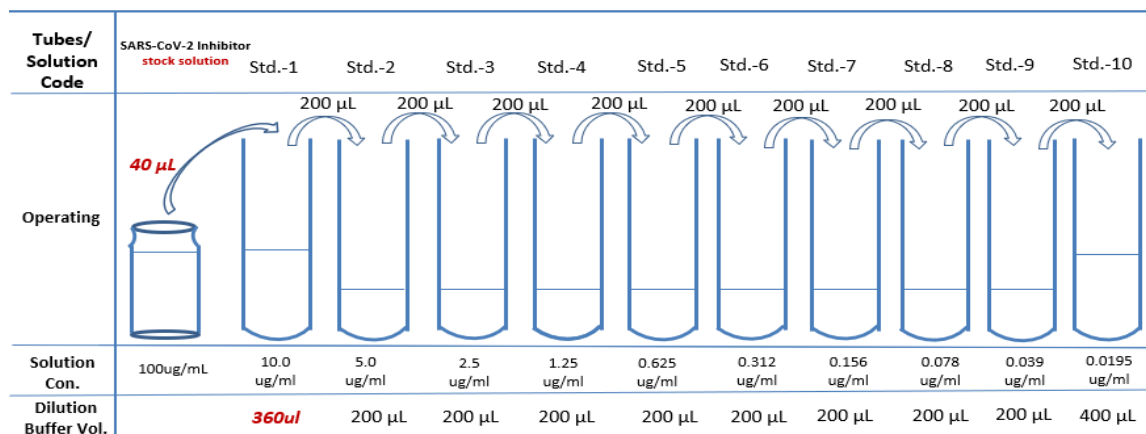
1.2 Preparation of HRP-SARS-CoV-2 Spike RBD(B.1.351) working fluid:

Dilute HRP-SARS-CoV-2 Spike RBD(B.1.351) rehydrated solution to 2.5 µg/ml with Dilution Buffer. The prepared working fluid should avoid light. Please prepare it for one-time use only.

2. Preparation of Standard Curve

Make serial dilutions of the SARS-CoV-2 Inhibitor as a Standard curve with Dilution Buffer as recommended in Figure 1.

FIGURE 1. PREPARATION OF 1:1 SERIAL DILUTIONS OF THE SARS-CoV-2 Inhibitor



3. Add Samples

- a. For SARS-CoV-2 Inhibitor Standard curve and samples: Add 50µL serially diluted SARS-CoV-2 Inhibitor Standard curve (Std.-1 to Std.-10) and samples to each well, then add 50 µL HRP-SARS-CoV-2 Spike RBD(B.1.351) working fluid to each well;
- b. For positive control wells, please add 50 µL Dilution Buffer, then add 50 µL HRP-SARS-CoV-2 Spike RBD(B.1.351) working fluid to each well;
- c. For blank Control wells, please add 100 µL Dilution Buffer (**No add** HRP-SARS-CoV-2 Spike RBD(B.1.351));

Please Seal the plate with microplate sealing film and incubate at 37°C for 1.0 h.

4. Washing

Remove the solution from the wells by aspiration. Add 300 µL 1 x Washing Buffer to each well, gently shake the plate for 30 s. Remove any remaining Washing Buffer by aspirating or decanting. Invert the plate and blot it against paper towels. Repeat the steps above for three times.

5. Substrate Reaction

Add 100 µL Substrate Solution to each well. Seal the plate with microplate sealing film and incubate at 37°C for 20 min. Avoid light.

6. Termination

Add 50 µL **Stop Solution** to each well, shake gently to mix.

Note: the color in the wells should change from blue to yellow.

7. Data Recording

Read the absorbance at 450 nm using UV/Vis microplate spectrophotometer.

Note: To reduce the background noise, subtract the value read at OD_{450nm} with the value read at OD_{630nm}.

CALCULATION OF RESULTS

To calibrate absorbance value obtained by the standard curve, the OD value of the sample to be measured is subtracted to the OD value of the blank control. The standard curve is plotted with the standard concentration as x-axis and the calibrated absorbance value as y-axis. Four parameters logistic or other statistical software are used to draw the standard

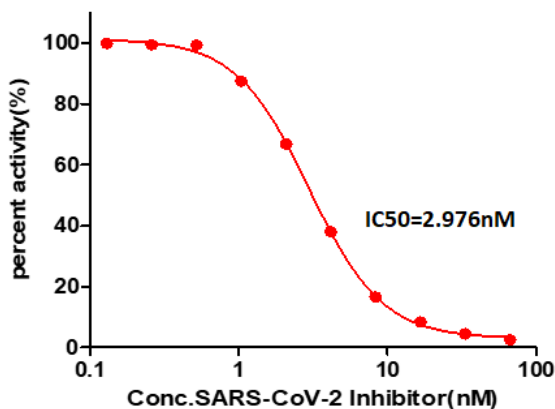
curve and calculate the sample concentration.

PRECAUTIONS

1. This kit is for research use only and is not for use in diagnostic or therapeutic procedures.
2. The kit should be used according to the instructions.
3. Do not mix reagents from different lots.
4. Bring all reagents and samples to room temperature (20°C-25°C) before use. If crystals have formed in buffer solution, warm to room temperature until the crystals have completely dissolved.
5. The kit should be stored at 2°C to 8°C.
6. Please prepare the working solution of each component according to the needs of the experiment. Except for 1×Washing Buffer, all prepared working solution is for one-time use and cannot be stored.

TYPICAL DATA

Serial dilutions of SARS-CoV-2 inhibitor (Catalog # EP109-C02) (1:1 serial dilutions, from 10.0 µg/mL to 0.0195 µg/mL) were added into HRP-SARS-CoV-2 Spike RBD(B.1.351). Background was subtracted from data points prior to log transformation and curve fitting. The following data is for reference only.



SARS-CoV-2 inhibitor Con.(µg/ml)	SARS-CoV-2 inhibitor Con.(nM)	Mean Abs(OD450nm)	percent activity(%)
0	0	2.969	100.0
0.020	0.130	2.966	99.9
0.039	0.260	2.955	99.5
0.078	0.521	2.952	99.4
0.156	1.042	2.599	87.5
0.313	2.083	1.987	66.9
0.625	4.167	1.131	38.1
1.250	8.334	0.493	16.6
2.500	16.668	0.250	8.4
5.000	33.335	0.134	4.5
10.000	66.670	0.078	2.6