

SARS-CoV-2 Inhibitor screening Kit

Pack Size: 96 tests / 480 tests

Catalog Number: EP-105

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

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

PRINCIPLE OF THE ASSAY

The newly identified 2019 novel coronavirus (SARS-CoV-2) has posed a serious threat to human health. Currently, however, there is no specific treatment for COVID-19. It is urgent to develop the SARS-CoV-2 inhibitors, for example vaccines and therapeutic antibodies and small molecular compounds to against COVID-19.

This inhibitor screening ELISA pair is designed to facilitate the identification and characterization of SARS-CoV-2 inhibitors. This assay employs a simple colorimetric ELISA platform, which measures the binding between immobilized SARS-CoV-2 S protein RBD and in-house developed biotinylated Human ACE2 protein. This product is uniquely suitable for rapid high-throughput screening of SARS-CoV-2 inhibitors. Briefly, we provide you with a biotinylated human ACE2 protein, a SARS-CoV-2 S protein RBD protein, an SARS-CoV-2 inhibitor (as method verified Reference), and Streptavidin-HRP reagent.

Your experiment will include 4 simple steps:

- a) Coat the plate with SARS-CoV-2 S protein RBD.
- b) Add 50ul biotinylated human ACE2 to coated plate.
- c) Add the reference or your molecule of interest.
- d) Add Streptavidin-HRP followed by TMB or other colorimetric HRP substrate.

Finally, the ability of your compound to inhibit S protein RBD: ACE2 binding will be determined by comparing OD readings among different experimental groups.

MATERIALS PROVIDED

TABLE 1. MATERIALS PROVIDED

Catalog	Components	Size (96 tests)	Size (480 tests)	Format	Storage
A010-214	SARS-CoV-2 S protein RBD	10	60	Powder	-20°C
A011-214	Biotinylated Human ACE2	10	10	Powder	-20°C
A003-214	Streptavidin-HRP	10	10	Powder	-20°C, avoid light
AC2-NA005	SARS-CoV-2 Inhibitor	20	50	Powder	-20°C

RECONSTITUTION

Reconstitute the provided lyophilized materials to stock solutions with PBS as recommended in **Table 2.1** and **Table 2.2**, Solubilize for 15 to 30 minutes at room temperature with occasional gentle mixing. **Avoid vigorous shaking or vortexing.**

The reconstituted stock solutions should be stored at -70°C. **It is recommended not to freeze thaw more than 3 times.**

To avoid surface adsorption loss and inactivation, the reconstituted protein must NOT be aliquoted to less than 5 µg per vial.

Note: Streptavidin-HRP stock solution should be protected from light.

TABLE 2.1. RECONSTITUTION METHODS FOR 96 TESTS

Catalog	Components	Size	Stock Solution Con.	Reconstitution Buffer and Vol.
A010-214	SARS-CoV-2 S protein RBD	10	100 µg/mL	100 µL PBS
A011-214	Biotinylated Human ACE2	10	100 µg/mL	100 µL PBS
A003-214	Streptavidin-HRP	10	50 µg/mL	200 µL PBS
AC2-NA005	SARS-CoV-2 Inhibitor	20	200 µg/mL	100 µL PBS

TABLE 2.2. RECONSTITUTION METHODS FOR 480 TESTS

Catalog	Components	Size	Stock Solution Con.	Reconstitution Buffer and Vol.
A010-214	SARS-CoV-2 S protein RBD	60	100 µg/mL	600 µL PBS
A011-214	Biotinylated Human ACE2	10	100 µg/mL	100 µL PBS
A003-214	Streptavidin-HRP	10	50 µg/mL	200 µL PBS
AC2-NA005	SARS-CoV-2 Inhibitor	50	200 µg/mL	250 µL PBS

SHIPPING AND STORAGE

All components are shipped in lyophilized state at room temperature. This product is stable after stored at:

- 1) Room temperature (RT) for 1 month in lyophilized state.
- 2) -20°C to -70°C for 1 year in lyophilized state.
- 3) -70°C for 6 months under sterile conditions after reconstitution.

MATERIALS OR INSTRUMENTS REQUIRED BUT NOT SUPPLIED (for 96 tests)

Note: ELISA Buffer is not included in this Inhibitor Screening Kit, please order ELISA Buffer Set (Cat. No. EBS-001) if you need in your assay. If not, It is recommended to prepare according to the following formula.

Coating Buffer 15 mmol/L sodium carbonate (Na_2CO_3), 35 mmol/L sodium hydrogen carbonate (NaHCO_3), pH 9.6, 12 mL is sufficient for 96 tests.

Wash Buffer PBS with 0.05% (v/v) Tween-20 (pH7.4), 500 mL is sufficient for 96 tests.

Blocking Buffer Wash Buffer with 2% (w/v) bovine serum albumin (BSA), 35 mL is sufficient for 96 tests.

Dilution Buffer Wash Buffer with 0.5% (w/v) bovine serum albumin (BSA), 50 mL is sufficient for 96 tests.

TMB Substrate Working Solution TMB Substrate Working Solution with 50 mM disodium hydrogen phosphate (Na_2HPO_4) and 25 mM citric acid, adjust pH to 5.5 with 1 M Sodium hydroxide (NaOH), 40 mg/mL TMB in Dimethyl Sulfoxide. For **each plate** dilute 125 μL substrate stock solution in 25 mL substrate dilution buffer and add 20 μL 5% H_2O_2 , mix well. 25 mL is sufficient for 96 tests.

Notes:

- 1) The TMB Substrate Working Solution should be freshly prepared and used within 15 minutes.
- 2) If you choose to use other commercially available ready-to-use TMB substrate solutions, you should follow the manufacturer's instruction.

Stop Solution 1 M sulfuric acid (aqueous), 6 mL is sufficient for 96 tests.

High binding surface 96-well microplate, clear flat bottom (Corning, Catalog # 42592)

Microplate sealing film

Pipettes and pipette tips

UV/Vis microplate spectrophotometer (absorbance 450 nm, correction wavelength set to 630 nm).

RECOMMENDED PROTOCOL

1. Preparation

Reconstitute and store all reagents as recommended.

2. Coating

- 1) Dilute **SARS-CoV-2 S protein RBD** stock solution (100 µg/mL) to 0.5 µg/mL with **Coating Buffer** to make **SARS-CoV-2 S protein RBD** working solution.
- 2) Please leave two wells uncoated for **No-Coating Control (Table 3)**.
- 3) Add 100 µL of **SARS-CoV-2 S protein RBD** working solution (0.5 µg/mL) to each well, seal the plate with microplate sealing film and incubate overnight (or 16 hours) at 4 °C.

3. Washing

Remove the remaining solution by aspiration, add 300 µL of **Wash buffer** to each well, gently tap the plate for 1 minute, remove any remaining **Wash Buffer** by aspirating or decanting, invert the plate and blot it against paper towels. **Repeat the wash step above for three times.**

4. Blocking

Add 300 µL **Blocking Buffer** to each well, seal the plate with microplate sealing film and incubate at 37 °C for 1.5 hours.

5. Washing

Repeat step 3. At meantime, you can start to prepare your **samples**.

6. Binding

- 1) Dilute **Biotinylated Human ACE2** stock solution (100 µg/mL) to 0.12 µg/mL with **Dilution Buffer** to make **Biotinylated Human ACE2** working solution.
- 2) For **No-Binding Control** wells, please add 50 µL **Dilution Buffer**.
- 3) For all other wells, please add 50 µL **Biotinylated Human ACE2** working solution.

7. Add Samples

- 1) Make series dilution of the samples as appropriate.
- 2) If you intend to use the provided **SARS-CoV-2 Inhibitor** as a reference (Ref.), you may dilute the inhibitor as recommended in **Figure 1**. And plate layout as recommended in **Figure 2**.
- 3) For **Positive control** wells, please add 50 µL Dilution Buffer.
- 4) For all other wells, Add 50 µL of sample solution to each well according to our recommendation (**Figure 2**) or your own plate setup.
- 5) Seal the plate with microplate sealing film and incubate at 37 °C for 1 hour.

8. Washing

Remove the remaining solution by aspiration, add 300 µL of **Wash buffer** to each well, gently tap the plate for 1 minute, remove any remaining **Wash Buffer** by aspirating or decanting, invert the plate and blot it against paper towels. **Repeat the wash step above for three times.**

9. Streptavidin-HRP Labeling

- 1) Dilute **Streptavidin-HRP** stock solution (50 µg/mL) to 0.1 µg/mL with **Dilution Buffer** to make **Streptavidin-HRP** working solution.
- 2) For all wells, add 100 µL **Streptavidin-HRP** working solution, seal the plate with microplate sealing film⁹

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and incubate at 37°C for 1 hour, **avoid light**.

10. Washing

Repeat step 8.

11. TMB Substrate Reaction

Add 200 µL **TMB Substrate Working Solution** to each well. Seal the plate with microplate sealing film and incubate at 37°C for 20 minutes, **avoid light**.

12. Termination

Add 50 µL **Stop Solution** to each well, and tap the plate gently for 3 minutes to allow thorough mixing.

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

13. Data Recording

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

***Note:** the plate may be read at 600 nm without adding 1 M sulfuric acid, but the Signal-to-Background ratio may be reduced.*

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

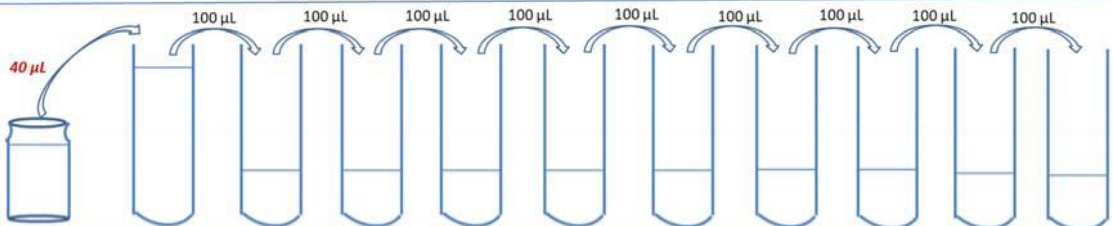
Tubes/ Solution Code	2019-nCoV inhibitor stock solution	Ref.-1	Ref.-2	Ref.-3	Ref.-4	Ref.-5	Ref.-6	Ref.-7	Ref.-8	Ref.-9	Ref.-10
Operating		100 µL	100 µL	100 µL	100 µL	100 µL	100 µL	100 µL	100 µL	100 µL	100 µL
Solution Con.	200 µg/mL	40 µg/mL	20 µg/mL	10 µg/mL	5 µg/mL	2.5 µg/mL	1.25 µg/mL	0.63 µg/mL	0.31 µg/mL	0.16 µg/mL	0.078 µg/mL
Dilution Buffer Vol.		200 µL	100 µL	100 µL	100 µL	100 µL	100 µL	100 µL	100 µL	100 µL	100 µL

FIGURE 2. PLATE LAYOUT

	1	2	3	4	5	6	7	8	9	10	11	12
A	Ref.-8	Ref.-8	Positive Ctrl.	Positive Ctrl.	...	...	...	...	...	...	...	...
B	Ref.-7	Ref.-7	No-binding Ctrl.	No-binding Ctrl.	...	...	...	...	...	...	...	...
C	Ref.-6	Ref.-6	No-coating Ctrl.	No-coating Ctrl.	...	...	...	...	...	...	...	...
D	Ref.-5	Ref.-5	Ref.-9	Ref.-9	...	...	...	...	...	...	...	...
E	Ref.-4	Ref.-4	Ref.-10	Ref.-10	...	...	...	...	...	...	...	...
F	Ref.-3	Ref.-3	...	...	...	...	...	...	...	...	...	...
G	Ref.-2	Ref.-2	...	...	...	...	...	...	...	...	...	...
H	Ref.-1	Ref.-1	...	...	...	...	...	...	...	...	...	...

TABLE 3. ASSAY PROTOCOL

Steps Code	Steps	Reagents & Instruments	Reaction Conditions	Samples	No-binding Control	No-coating Control	Positive Control
1	Preparation	N/A	N/A	N/A	N/A	N/A	N/A
2	Coating	SARS-CoV-2 S protein RBD Working Solution	4°C for overnight	100 µL	100 µL	—	100 µL
3	Washing	Wash Buffer	Wash for 3 times	300 µL	300 µL	300 µL	300 µL
4	Blocking	Blocking Buffer	37°C for 1.5 hours	300 µL	300 µL	300 µL	300 µL
5	Washing	Wash Buffer	Wash for 3 times	300 µL	300 µL	300 µL	300 µL
6	Binding	Biotinylated Human ACE2 Working Solution	N/A	50 µL	—	50 µL	50 µL
		Dilution Buffer		—	50 µL	—	—
6	Add Samples	Samples	Incubate at 37°C for 1 hour	50 µL	50 µL	50 µL	
		Dilution Buffer		—	—	—	50 µL
8	Washing	Wash Buffer	Wash for 3 times	300 µL	300 µL	300 µL	300 µL
9	Streptavidin-HRP Labeling	Streptavidin-HRP Working Solution	37°C for 1 hours	100 µL	100 µL	100 µL	100 µL
10	Washing	Wash Buffer	Wash for 3 times	300 µL	300 µL	300 µL	300 µL
11	TMB Substrate Reaction	TMB Substrate Working Solution	37°C for 20 minutes	200 µL	200 µL	200 µL	200 µL
12	Stop the Reaction	Stop Solution	Mix by gentle tapping for 3 minutes	50 µL	50 µL	50 µL	50 µL
13	Data Recording	UV/Vis spectrophotometer	Measure absorbance at 450 nm, with the correction wavelength set at 600 nm				

Note for TABLE3:

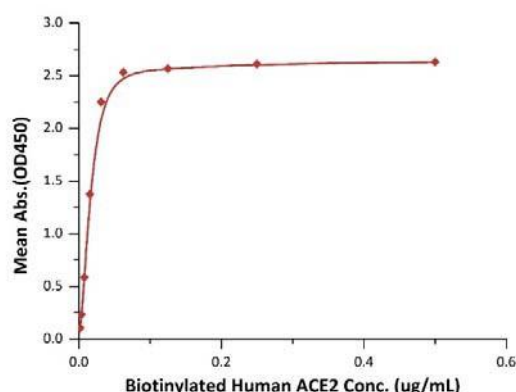
- 1) **Samples:** Your samples of interest.
- 2) **No-Binding Control:** Reaction without **Biotinylated Human ACE2** added. The absorbance should be around 0.05(< 0.1) at 450 nm.
- 3) **No-Coating Control:** Reaction without **SARS-CoV-2 S protein RBD** coated on the wells. The absorbance should be around 0.05(< 0.1) at 450 nm.
- 4) **Positive Control:** Determined the max value in 450 nm absorbance, when out of inhibitors.
- 5) It is recommended that all samples, controls and references should be done in duplicates.

METHOD VERIFICATION

● HUMAN ACE2 [BIOTINYLATED]: SARS-CoV-2 S PTOTEIN RBD IN THE ABSENCE OF INHIBITORS

Immobilized **SARS-CoV-2 S** protein RBD at 0.5 µg/mL (100 µL/well) can bind **Biotinylated Human ACE2** with a linear range of 0.001-0.06 µg/mL when detected by **Streptavidin-HRP**. Background was subtracted from data points before curve fitting.

FIGURE 3. BINDING OF BIOTINYLATED HUMAN ACE2 TO IMMOBILIZED SARS-CoV-2 S PTOTEIN RBD IN A FUNCTIONAL ELISA ASSAY

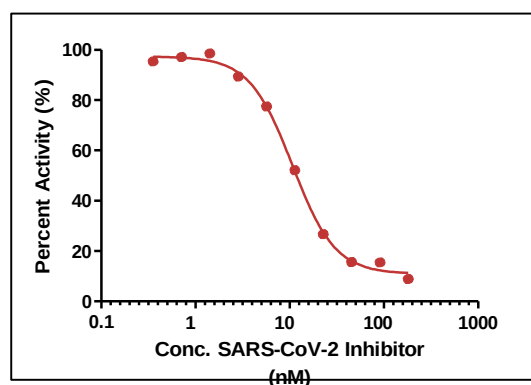


Biotinylated Human ACE2 (µg/mL)	Mean Abs.(OD450)
0.5	2.63
0.25	2.61
0.125	2.57
0.0625	2.53
0.03125	2.25
0.015625	1.37
0.0078125	0.59
0.00390625	0.23
0.001953125	0.10

● INHIBITION OF HUMAN ACE2 [BIOTINYLATED]: SARS-CoV-2 S PTOTEIN RBD BINDING BY 2019-NCOV INHIBITOR

Serial dilutions of **SARS-CoV-2 inhibitor** (Catalog # AC2-NA005) (1:2 serial dilutions, from 20 µg/mL to 0.039063 µg/mL) were added into **Biotinylated ACE2: SARS-CoV-2 S protein RBD** binding reactions. The assay was performed according to the above described protocol. Background was subtracted from data points prior to log transformation and curve fitting.

FIGURE 4. INHIBITION OF HUMAN ACE2 [BIOTINYLATED]: SARS-CoV-2 S PTOTEIN RBD BINDING BY SARS-CoV-2 INHIBITOR



SARS-CoV-2 inhibitor Con. (µg/mL)	SARS-CoV-2 inhibitor Con. (nM)	Log[SARS-CoV-2 inhibitor Con. (nM)]	Mean Abs. (OD450)	Percent Activity (%)
0	0		2.600	100.00
0.039063	0.3515625	-0.45	2.483	95.50
0.078125	0.703125	-0.15	2.527	97.19
0.15625	1.40625	0.15	2.564	98.62
0.3125	2.8125	0.45	2.331	89.65
0.625	5.625	0.75	2.030	78.08
1.25	11.25	1.05	1.389	53.42
2.5	22.5	1.35	0.745	28.65
5	45	1.65	0.465	17.88
10	90	1.95	0.460	17.69
20	180	2.26	0.293	11.27

TROUBLESHOOTING GUIDE

Problem	Possible Cause	Solutions
Signal of positive control is weak or abnormal	Reconstituted protein be aliquoted to less than 5 µg per vial	✧ Reconstituted protein must NOT be aliquoted to less than 5 µg per vial.
	The working solution not be prepared immediately before use	✧ The working solution should be prepared immediately before use and should not be stored.
	Biotinylated ACE2, SARS-CoV-2 S protein RBD or Streptavidin - HRP may have lost activity	✧ Make sure your proteins are aliquoted into single-use aliquots. ✧ Increase the time of reaction or increase the protein concentration may help in case the protein activity is decreased over time.
	Errors in instrument settings	✧ Please check instrument setting.
	Substrate Stock Solution is outdated; Incubation temperature is incorrect; Incubation time is not sufficient; Repeated freeze-thaw cycles;	✧ Make sure the Substrate Stock Solution is working. ✧ Use proper incubation time and temperature.
	Pipetting errors	✧ Make sure that the pipette is calibrated and working properly.
High background	Insufficient washing or blocking	✧ Be sure the blocking step is performed. ✧ Increase number of washes and the volume Wash Buffer used. ✧ Increase Tween-20 concentration to 0.1% in Wash Buffer . ✧ Make sure Streptavidin-HRP is diluted in Blocking Buffer .
	Sample solvent contains inhibiting factors	✧ Run a negative control assay with the solvent alone. ✧ Maintain DMSO level at <1%. Increase protein incubation time.
	Contamination	✧ Make sure buffers and samples are prepared, used and stored correctly.
	The TMB Substrate Working Solution is not fresh	✧ TMB Substrate Working Solution must be used within 15 minutes after preparation.
Colorimetric signal is erratic	Inconsistent pipetting or dilution methods	✧ Make sure pipettors are functioning properly and use a multichannel pipettor if possible. ✧ Use master mixes to minimize errors. ✧ Run duplicates for all tests.
	TMB Substrate Working Solution is not completely mixed with the reaction solution	✧ Make sure that TMB Substrate Working Solution is adequately mixed with the reaction solution.
	Bubbles in the wells	✧ Tap plate gently to disperse bubbles.
	Signal is too high	✧ The concentration of the samples should be adjusted to achieve optimal reading. ✧ Decrease colorimetric HRP substrate incubation time.
Inadequate color development	Incomplete removal of residual buffers during previous steps	✧ Wells should appear dry after aspiration.
	Problems with conjugate or color reagents	✧ Color should appear immediately after the reagent is added. Make sure no contamination or residual buffers in the wells before you start the color development process.