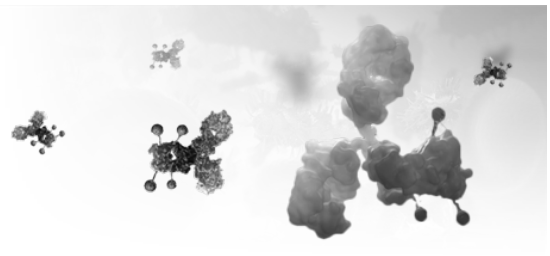


SARS-CoV-2 (2019-nCoV) Inhibitor Screening ELISA Kit (DEIA-WZ021)

This product is for research use only and is not intended for diagnostic use.

PRODUCT INFORMATION

Size	N/A
Intended Use	The kit applies for the screening of SARS-CoV-2 (2019-nCoV) inhibitors.
Principle of the Assay	The principle ofthe kit is competitive ELISA. The SARS-CoV-2 (2019-nCoV) Inhibitor in the samples competes with ACE2-His to combine with immobilized SARS-CoV-2 S Protein RBD. The signal color becomes lighter as the content of SARS-CoV-2 Inhibitor increases.
Reagents And Materials Provided	SARS-CoV-2 Inhibitor: 40 µL Human ACE2 (His Tag): 1 vial Anti-His Tag Ab (HRP): 20 µL Microplate: Pre-coated with SARS-CoV-2 (2019-nCoV) Spike RBD-mFc Recombiant Protein: 1 Plate 20 × Dilution Buffer: 5 mL 20 × Wash Buffer: 25 mL Color Reagent A: 12.5 mL Color Reagent B: 12.5 mL Stop Solution: 8 mL

Storage

	Unopened	Opened
SARS-CoV-2 Inhibitor	- 80℃, 6 months	- 80℃ Please used it in 2 weeks.
Human ACE2 (His Tag)	- 80℃, 6 months	After reconstitution, - 80℃, 1 months Recommend to aliquot the protein. Avoid repeated freeze-thaw cycles.
Anti-His Tag Ab (HRP)	2 - 8℃, 6 month. Protect from light.	2 - 8℃. Protect from light. Please used it in 2 weeks.
Microplate	2 - 8℃, 3 months	2 - 8℃, 1 month Return unused strips to the foil pouch containing the desiccant pack and reseal along entire edge of zip-seal.
20 ×Dilution Buffer	2 - 8℃, 6 months	2 - 8℃, 1 month (Unmixed Color Reagent A & B)
20 ×Wash Buffer		
Color Reagent A		
Color Reagent B		
Stop Solution		

Please store the reagentsas above conditions upon receiving, and used as soon as possible after opening.

Reagent Preparation

Bring all reagents to room temperature before use.If crystals have formed in buffer solution, warm to room temperature and mix gently until the crystals have completely dissolved.

Wash Buffer - Prepare 1× wash buffer by adding 20 mL of Wash Buffer Concentrate to deionized or distilled water to prepare 400 mL of Wash Buffer.

Dilution Buffer - Prepare 1× dilution buffer by adding 5 mL of Dilution Buffer Concentrate to deionized or distilled water to prepare 100 mL of Dilution Buffer.

Human ACE2 (His Tag) - Pipette 1 mL of Dilution Buffer into the vial, which containing lyophilized Human ACE2 (His Tag) recombinant protein, then 1:40 dilute with Dilution Buffer before use.

Anti-His Tag Ab (HRP) - Centrifuge at 10,000 x g for 20 seconds. 1:3000 dilute with Dilution Buffer before use.

Substrate Solution - Color Reagents A and B should be mixed together in equal volumes within 15 minutes of use. Protect from light. 200 µL of the resultant mixture is required per well. Take care not to contaminate the Color Reagent. If the mixed color reagent is blue. DO NOT USE.

Assay Procedure

Bring all reagents and samples to room temperature before use. It is recommended that all samples and standards be assayed in duplicate.

1. Prepare all reagents and samples as directed in the previous sections.

2. Remove unused microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, and reseal.

3. Wash each well three times with Wash Buffer (300 µL/well) using multi-channel pipette, manifold dispenser or autowasher. Complete removal of liquid at each step is essential to good performance. Remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against clean paper towels.

4. Add 100 µL Human ACE2 (His Tag) to pre-coated plate, then add 100 µL of triple series diluted SARS-CoV-2 Inhibitor (add 1 × Dilution Buffer to control well) or your test sample per well. The maximum concentration of the SARS-CoV-2 Inhibitor is 80 nM (Pipette 11.32 µL of SARS-CoV-2 Inhibitor into 1 mL 1×Dilution Buffer) . Then Pipette 300 µL of 80 nM SARS-CoV-2 Inhibitor into 600 µL 1 ×Dilution Buffer. And so on, dilute 10 series of concentrations. Ensure reagent addition is uninterrupted and completed within 15 minutes. Cover/seal the plate and incubate for 1 hours at room temperature.

5. Repeat the aspiration/wash as in Step 3.

6. Add 100 µL of Anti-His Tag Antibody(HRP)in working concentration to each well. Cover/seal the plate and incubate for 1 hour at room temperature.

7. Repeat the aspiration/wash as in Step 3.

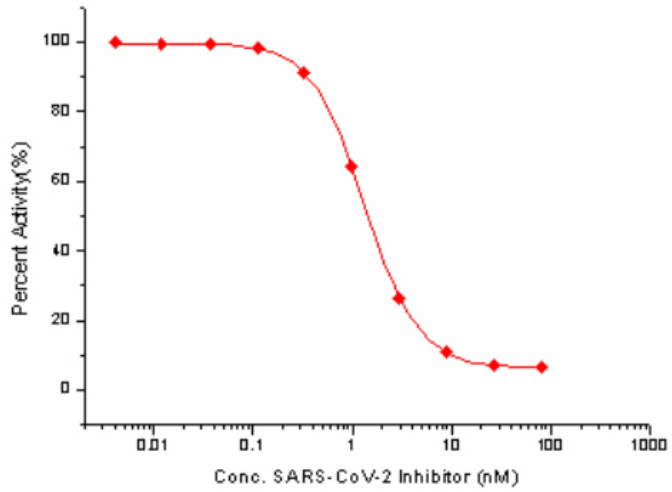
8. Add 200 µL of Substrate Solution to each well. Incubate for 15 minutes at room temperature. Protect from light.

9. Add 50 µL of Stop Solution to each well. If color change does not appear uniform, gently tap the plate to ensure thorough mixing.

10. Determine the optical density of each well within 15 minutes, using a microplate reader set to 450 nm.

Typical Standard Curve

SARS-CoV-2 Inhibitor concentration(nM)	mean value OD_{450nm}	percent activity(%)
0	1.126	
0.004	1.125	99.9
0.012	1.118	99.3
0.037	1.120	99.5
0.110	1.107	98.3
0.329	1.026	91.1
0.988	0.725	64.4
2.963	0.297	26.4
8.889	0.122	10.8
26.667	0.079	7
80.000	0.074	6.6



The inhibition curve of SARS-CoV-2 Inhibitor to the binding between RBD and ACE2, IC_{50}=1.34 nM.

Precautions

1. This kit is for research use only and is not for use in diagnostic or therapeutic procedures.
2. The kit should not be used beyond the expiration date.
3. Do not mix reagents from different lots.
4. The kit is designed and tested to detect the specific targets and samples shown in the manual. The use of this kit for other purpose should be verified carefully by the end user.
5. The Stop Solution provided with this kit is an acid solution. Take care when using the reagent to avoid the risks.
6. All biological materials should be handled and discarded as potentially hazardous following local laws and regulations.
7. Personal protective equipments such as lab coats, gloves, surgical masks and goggles are necessary in experiments for safety reasons.