CoviDrop SARS-CoV-2 Spike-ACE2 Binding Inhibitor Screening Fast Kit

Base Catalog # D-1004

PLEASE READ THIS ENTIRE USER GUIDE BEFORE USE

Uses: The CoviDrop™ SARS-CoV-2 Spike-ACE2 Binding Inhibitor Screening Fast Kit is designed for screening SARS-CoV-2-ACE2 binding inhibitors using purified SARS-CoV-2 spike protein and ACE2 protein in a fast and high throughput format.

Input Material: Input materials can be small molecule compounds, antibodies, and other biological molecules that inhibit or interfere with the binding of SARS-CoV-2 to ACE2.

Internal Control: An ACE2 antibody as the inhibition control is provided in this kit for the quantification of sample ACE2 binding inhibition. Because binding inhibition of SARS-CoV-2 spike to ACE2 can vary with different inhibitors, it is advised to run replicate samples to ensure that the signal generated is validated.

Precautions: To avoid cross-contamination, carefully pipette the sample or solution into the strip wells. Use aerosol-barrier pipette tips and always change pipette tips between liquid transfers. Wear gloves throughout the entire procedure. In case of contact between gloves and sample, change gloves immediately.

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KIT CONTENTS

Component	48 Assays Cat. #D-1004-48	96 Assays Cat. #D-1004-96	Storage Upon Receipt
WB (10X Wash Buffer)	14 ml	28 ml	4°C
AB (Assay Buffer)	6 ml	12 ml	RT
ACE2 (His-ACE2 protein, 1 mg/ml)*	4 µl	8 µl	–20°C
BIC (Binding Inhibition Control, 1 mg/ml)*	5 µl	10 µl	–20°C
BDS (Binding Detection Solution, 2000X)*	4 µl	8 µl	–20°C
DS (Developer Solution)	5 ml	10 ml	4°C
SS (Stop Solution)	5 ml	10 ml	RT
8-Well Assay Strips (With Frame)	6	12	4°C

**Spin the solution down to the bottom prior to use.*

SHIPPING & STORAGE

The kit is shipped in two parts: the first part at ambient room temperature and the second part on frozen ice packs at 4°C.

Upon receipt: (1) Store **ACE2**, **BIC** and **BDS** at -20°C away from light; (2) Store **WB**, **DS** and **8-well assay strips** at 4°C away from light; (3) Store all remaining components (**AB** and **SS**) at room temperature away from light.

All components of the kit are stable for 6 months from the date of shipment, when stored properly.

Note: *(1) Check if **WB** (10X Wash Buffer) contains salt precipitates before use. If so, warm (at room temperature or 37°C) and shake the buffer until the salts are re-dissolved. (2) Transfer the amount of **DS** (Developer Solution) required into a secondary container (tube or vial) before adding **DS** into the assay wells in order to avoid contamination. Check if a blue color is present in **DS** before each use, as this would indicate contamination of the solution and should not be used.*

MATERIALS REQUIRED BUT NOT SUPPLIED

- ☐ Adjustable pipette or multiple-channel pipette
- ☐ Multiple-channel pipette reservoirs
- ☐ Aerosol resistant pipette tips
- ☐ Microplate reader capable of reading absorbance at 450 nm
- ☐ 1.5 ml microcentrifuge tubes
- ☐ Incubator for 37°C incubation
- ☐ Distilled water
- ☐ Inhibitor of interest
- ☐ Parafilm M or aluminium foil

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GENERAL PRODUCT INFORMATION

Quality Control: Each lot of this product is tested against predetermined specifications to ensure consistent product quality. EpigenTek guarantees the performance of all products in the manner described in our product instructions.

Product Warranty: If this product does not meet your expectations, simply contact our technical support unit or your regional distributor. We also encourage you to contact us if you have any suggestions about product performance or new applications and techniques.

Safety: Suitable lab coat, disposable gloves, and proper eye protection are required when working with this product.

Product Updates: EpigenTek reserves the right to change or modify any product to enhance its performance and design. The information in this User Guide is subject to change at any time without notice. Be sure to use the latest User Guide for this kit which can be accessed online at www.epigentek.com/datasheet.

Usage Limitation: This product is for research use only and is not intended for diagnostic or therapeutic application.

Intellectual Property: This product and methods of use contain proprietary technologies by EpigenTek.

A BRIEF OVERVIEW

COVID-19 is an infectious disease caused by SARS-CoV-2, a new member of the same coronavirus family that caused SARS and MERS. Entry of SARS-CoV-2 into human host cells occurs through binding of surface unit S1 of its spike protein to the cell receptor angiotensin-converting enzyme 2 (ACE2). ACE2 is ubiquitous and widely expressed in the lung, heart, kidney, vessels, brain, gut, and testis at different levels. ACE2 is also mostly bound to cell membranes and normally less present in circulation [1]. It was found that increased ACE2 expression in nasal epithelium and lung is associated with higher rates of SARS-CoV-2 infection and severity of COVID-19, respectively [2,3]. A new observation indicates that increased ACE2 concentration in circulation may be associated with increased incidence and fatality rate of COVID-19 [4]. In addition, SARS-CoV-2 invasion degrades ACE2 on cell membranes and may increase soluble ACE2 concentration in circulation [1].

The importance of screening inhibitors of SARS-CoV-2-ACE2 binding is emphasized by the complicated SARS-CoV-2-ACE2 interaction under normal (healthy) and abnormal (pre-existing diseases and viral infection) conditions, the current COVID-19 pandemic, and its possible resurgence.

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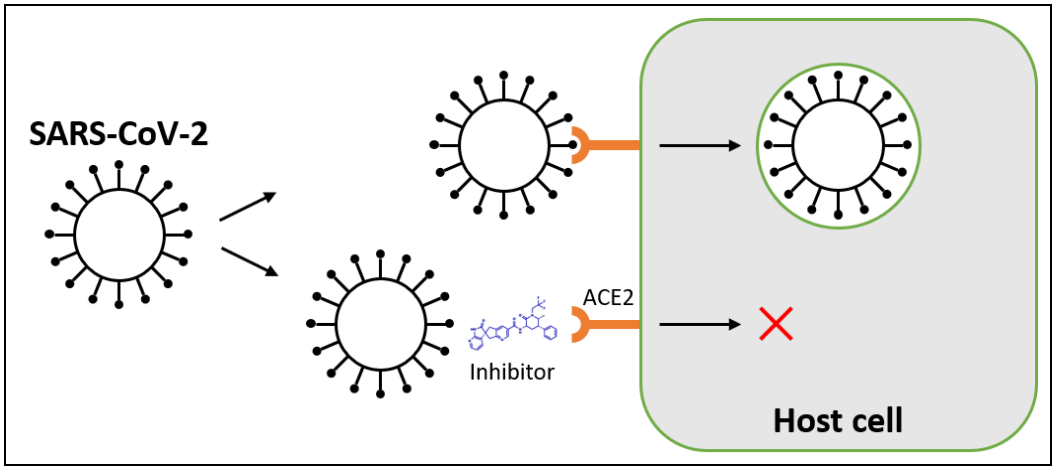

Fig 1. SARS-Cov-2 binding to ACE2 and entry into cells

Thus, such inhibitor screening would help in the development of effective drugs, vaccine, and therapeutic antibodies targeting COVID-19.

There are a few methods available for screening inhibitors of SARS-CoV-2-ACE2 binding. However, nearly all of these assay methods are time consuming (2 days). To address this issue, EpigenTek has developed and offers the CoviDrop™ SARS-CoV-2 Spike-ACE2 Binding Inhibitor Screening Fast Kit. The kit has the following advantages and features:

- **Fast:** Colorimetric assay with easy-to-follow steps for convenience and speed. The entire procedure can be finished within 1 hour and 30 minutes.
- **Robust:** Innovative kit composition enables background signals to be extremely low and allows the assay to be simple, accurate, reliable, and consistent.
- **Sensitive and Specific:** The activity can be detected from as low as 0.1 ng of ACE2 binding and specific for SARS-CoV-2-ACE2 binding, which is suitable for detecting inhibitory effects of various inhibitors, specifically against SARS-CoV-2, including small molecule chemicals, therapeutic peptide or antibodies, and other biological inhibitors.
- **Quantitative:** The assay standard is included, which allows the bound ACE2 to be quantified.
- **Flexible**: Strip-well microplate format makes the assay flexible for manual or high throughput analysis.

References:

1. *Verdecchia P et al: Eur J Intern Med. 76: 14–20, 2020*
2. *Bunyavanich S et al: JAMA. Published online May 20, 2020.*
3. *Sama IZ et al: Eur Heart I, 41: 1810–1817, 2020.*
4. *Pinto BGG et al: medRxiv. Published online March 27, 2020.*

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PRINCIPLE & PROCEDURE

The CoviDrop™ SARS-CoV-2 Spike-ACE2 Binding Inhibitor Screening Fast Kit contains all reagents necessary for screening various inhibitors of SARS-CoV-2 spike-ACE2 binding activity or inhibition. In this assay, a SARS-CoV-2 spike protein is stably pre-coated onto microplate wells. His-tagged ACE2 is bound to the coated spike protein in the presence or absence of inhibitors. The amount of the bound ACE2, which is proportional to ACE2 inhibition intensity, is then recognized by the Binding Detection Solution containing anti-His antibody and measured through an ELISA-like reaction by reading the absorbance in a microplate spectrophotometer at a wavelength of 450 nm. The binding activity of ACE2 is proportional to the optical density intensity measured. The more the ACE2 binding is inhibited, the lower the OD intensity is.

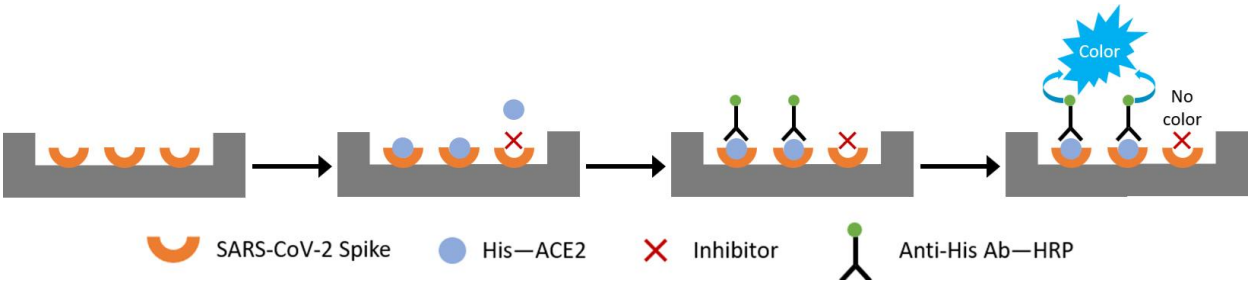

Fig 2. Schematic procedure of the CoviDrop™ SARS-CoV-2 Spike-ACE2 Binding Inhibitor Screening Fast Kit

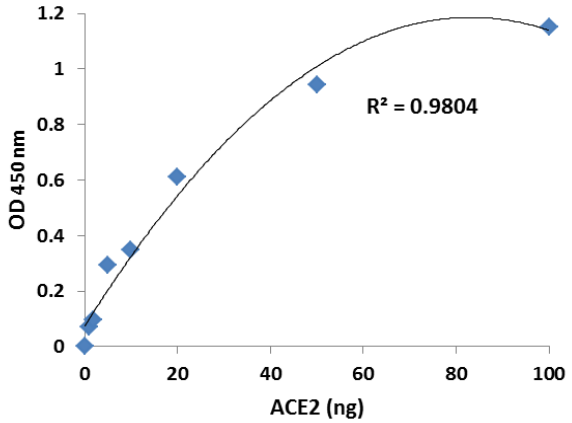

Fig 3. Illustrated dose-response of SARS-CoV-2-ACE2 binding assayed with the CoviDrop™ SARS-CoV-2 Spike-ACE2 Binding Inhibitor Screening Fast Kit.

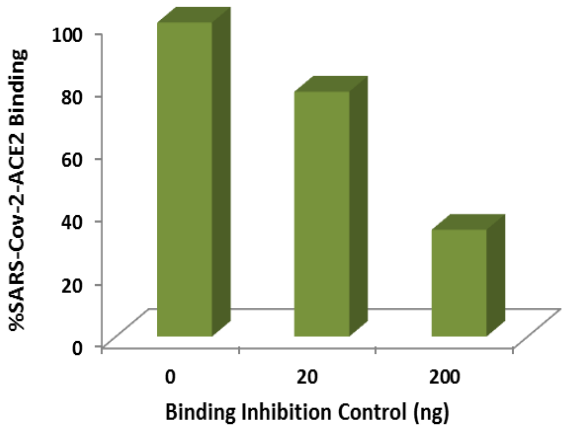

Fig 4. SARS-CoV-2-ACE2 binding inhibition by Binding Inhibition Control detected with the CoviDrop™ SARS-CoV-2 Spike-ACE2 Binding Inhibitor Screening Fast Kit.

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ASSAY PROTOCOL

1. Buffer Solution & Preparation

a. Prepare **Diluted WB (**1X Wash Buffer):

48-Assay Kit: Add 13 ml of **WB** (10X Wash Buffer) to 117 ml of distilled water and adjust pH to 7.2-7.5.

96-Assay Kit: Add 26 ml of **WB** (10X Wash Buffer) to 234 ml of distilled water and adjust pH to 7.2-7.5.

This **Diluted WB** (1X Wash Buffer) can now be stored at 4°C for up to six months.

b. Prepare **Diluted BDS (**Binding Detection Solution):

Dilute **BDS (**Binding Detection Solution) with **Diluted WB** (1X Wash Buffer) at a ratio of 1:2000 (i.e., add 1 µl of **BDS** to 2000 µl of **Diluted WB**). About 50 µl of this **Diluted BDA** will be required for each assay well.

c. Prepare working **ACE2** (His-ACE2 Protein) solution:

Dilute **ACE2** (His-ACE2 Protein) with **AB** (Assay Buffer) at 1:1000 ratio (ex: 5 µl of **ACE2** + 5000 µl of **AB**). The final concentration of working **ACE2** (His-ACE2 Protein) should be 1.0 µg/ml.

d. Prepare **Diluted BIC** (Binding Inhibition Control):

Dilute the **BIC** (Binding Inhibition Control) to 100 µg/ml (strong BIC) and 10 µg/ml (weak BIC), respectively with **AB** (Assay Buffer) according to the following chart:

Tube	BIC (Binding Inhibition Control) (1 mg/ml**)**	Diluted BIC (100 ug/ml)	AB	Resulting Concentration
1	1.0 µl		9 µl	100 µg/ml
2		1.0 µl	9 µl	10 µg/ml

Note*: (1) Keep each of diluted solutions except* ***Diluted WB*** *(1X Wash Buffer) on ice until use. Any remaining diluted solutions other than* ***Diluted WB*** *should be discarded if not used within the same day.*

2. Binding and Inhibition Reaction

a. Predetermine the number of strip wells required for your experiment. It is advised to run replicate samples (including blank and positive control) to ensure that the signal generated is validated. Carefully remove un-needed strip wells from the plate frame and place them back in the bag (seal the bag tightly and store at 4°C).

b. Blank Wells: Add 50 µl of **AB** (Assay Buffer) per well.

c. Positive Control Wells: Add 48 µl of **AB** (Assay Buffer) and 2 µl of **Diluted BIC (**Binding Inhibition Control) at concentrations of 100 µg/ml and 10 µg/ml, respectively per well (see the chart in Step 1d).

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d. Sample Wells Without Inhibitor: Add 50 µl of **AB** (Assay Buffer) per well.

e. Sample Wells With Inhibitor: Add 45 µl of **AB** (Assay Buffer), and 5 µl of inhibitor solution per well.

f. Tightly cover the strip-well microplate with Parafilm M or aluminum foil to avoid evaporation, and incubate at room temperature for 15 min.

Note: *(1) Follow suggested well setup diagrams; (2) The concentration of inhibitors to be added into the sample wells can be varied. However, the final concentration of the inhibitors before adding to the wells should be prepared with* **AB** (Assay Buffer), *at a 1:10 ratio (e.g., add 0.5 µl of inhibitor to 4.5 µl of* ***AB****), so that the original solvent of the inhibitor can be reduced to 1% of the reaction solution or less.*

g. After 15 min incubation, add 50 µl of the working **ACE2** (His-ACE2 Protein) solution to each well except blank wells. Add 50 µl of **AB** (Assay Buffer) to blank wells.

h. Tightly cover the strip-well microplate again with Parafilm M or aluminum foil to avoid evaporation, and incubate at 37°C for 45 min.

i. Remove the reaction solution from each well. Wash each well three times with 150 µl of **Diluted WB (**1X Wash Buffer) each time. This can be done by simply pipetting **Diluted WB** in and out of the wells.

3. Binding Inhibition Detection

a. Add 50 µl of the **Diluted BDS (**Binding Detection Solution) to each well, then carefully cover with Parafilm M or aluminum foil and incubate at room temperature for 20 min.

b. Remove the **Diluted BDS (**Binding Detection Solution) from each well.

c. Wash each well with 150 µl of the **Diluted WB (**1X Wash Buffer) each time for five times.

Note*: Ensure any residual wash buffer in the wells is thoroughly removed as much as possible at each wash step.*

d. Add 100 µl of **DS** (Developer Solution) to each well and incubate at room temperature for 1 to 10 min away from direct light. Monitor color change in the sample wells and control wells. The **DS** solution will turn blue in the presence of sufficient ACE2 protein.

e. Add 100 µl of **SS** (Stop Solution) to each well to stop enzyme reaction when the color in the Untreated control l wells turns medium blue. Mix the solution by gently shaking the frame and wait 2 min to allow the color reaction to be completely stopped. The color will change to yellow after adding **SS** and absorbance should be read on a microplate reader within 2 to 10 min at 450 nm with an optional reference wavelength of 655 nm.

Note: *(1) Most microplate readers have the capability to carry out dual wavelength analysis and will automatically subtract the reference wavelength absorbance from the test wavelength absorbance. If your plate reader does not have this capability, the plate can be read twice – once at 450 nm and once at 655 nm. Then manualy subtract the 655 nm ODs from 450 nm ODs; (2) If the stripwell microplate frame does not fit in the microplate reader, transfer the solution to a standard 96-well microplate.*

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4. Binding Inhibition Calculation

a. Calculate the average duplicate readings for sample wells and blank wells.

b. Calculate binding activity inhibition using the following formula:

$$\textit{Binding Inhibition \%} = \left[1 - \frac{\textit{Inhibitor Sample OD} - \textit{Blank OD}}{\textit{No Inhibitor Sample OD} - \textit{Blank OD}}\right] \times 100\%$$

SUGGESTED WORKING BUFFER AND SOLUTION SETUP

Table 1. Approximate amounts of required buffers and solutions for defined assay wells, based on the protocol.

Reagents	16 wells (2 strips)	48 wells (6 strips)	96 wells (12 strips)
Diluted WB (1X Wash Buffer)	25 ml	75 ml	150 ml
AB (Assay Buffer)	2 ml	6 ml	12 ml
Working ACE2 (His-ACE2 Protein)	1 µl	2 µl	2-4 µl
Diluted BDS (Binding Detection solution)	1 ml	3 ml	6 ml
DS (Developer Solution)	1.6 ml	5 ml	10 ml
SS (Stop Solution)	1.6 ml	5 ml	10 ml

SUGGESTED STRIPWELL SETUP

Table 2. The suggested strip-well plate setup for the binding activity assay in a 48-assay format (in a 96-assay format, Strips 7 to 12 can be configured as Sample). The controls and samples can be measured in duplicates.

Well #	Strip 1	Strip 2	Strip 3	Strip 4	Strip 5	Strip 6
A	Blank	Blank	Sample	Sample	Sample	Sample
B	BIC Strong	BIC Strong	Sample	Sample	Sample	Sample
C	BIC Weak	BIC Weak	Sample	Sample	Sample	Sample
D	Sample	Sample	Sample	Sample	Sample	Sample
E	Sample	Sample	Sample	Sample	Sample	Sample
F	Sample	Sample	Sample	Sample	Sample	Sample
G	Sample	Sample	Sample	Sample	Sample	Sample
H	Sample	Sample	Sample	Sample	Sample	Sample

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TROUBLESHOOTING

Problem	Possible Cause	Suggestion
No signal or weak signal in untreated sample wells	Reagents are added incorrectly.	Check if reagents are added in the proper order with the right amount, and if any steps in the protocol may have been omitted by mistake.
	Incubation time and temperature are incorrect.	Ensure the incubation time and temperature described in the protocol are followed correctly.
	Incorrect absorbance reading.	Check if appropriate absorbance wavelength (450 nm filter) is used.
	Kit was not stored or handled properly.	Ensure all components of the kit were stored at the appropriate temperature, and the cap is tightly closed after each opening or use.
High background present in the blank wells	Insufficient washing of wells.	Check if washing recommendations at each step is performed according to the protocol.
	Contaminated by sample or positive control.	Ensure the well is not contaminated from adding sample or positive control accidentally or from using contaminated tips.
	Over development of color.	Decrease the development time in Step 3d before adding **SS** (Stop Solution) in Step 3e.
Large variation between replicate wells	Color reaction is not evenly stopped due to an inconsistency in pipetting time.	Ensure **DS** (Developer Solution) and **SS (**Stop Solution) are added at the same time between replicates or otherwise maintain a consistent timing in between each addition of solutions.
	Color reaction is not evenly stopped due to an inconsistent order of adding solutions.	Ensure all solutions, particularly **DS** (Developer Solution) and **SS** (Stop Solution), are added in the same order each time as all other solutions.
	The solutions are not evenly added due to inconsistency in pipetting volume.	Ensure the solution in each pipette tip is equal in the multi-channel pipette. Equilibrate the pipette tip in any solution before using them. Ensure the solutions, especially those with small volumes (e.g., 1 ul), are completely added into the wells.

RELATED PRODUCTS

SARS-CoV-2 Serological Detection

D-1001 Seroflash™ SARS-CoV-2 IgM/IgG Antibody Detection Kit
D-1002 SeroFlash™ SARS-CoV-2 IgG/IgM ELISA Fast Kit
D-1005 CoviDrop™ SARS-CoV-2 Spike-ACE2 Binding Activity/Inhibition Assay Kit