CoviDrop™ SARS-CoV-2 Spike-ACE2 Binding Activity/Inhibition Assay Kit

Base Catalog # D-1005

PLEASE READ THIS ENTIRE USER GUIDE BEFORE USE

Uses: The CoviDrop™ SARS-CoV-2 Spike-ACE2 Binding Activity/Inhibition Assay Kit is designed for measuring SARS-CoV-2-ACE2 binding activity and inhibition using various human samples so that the functional ACE2 binding level from different cells/tissues in different individuals can be identified. The samples can be used immediately or stored at proper conditions for future use.

Input Material: Input materials can be cell/tissue extracts, serum, plasma, swab samples, and various body fluids.

Internal Control: An ACE2 protein as the positive control (assay standard) is provided in this kit for the quantification of sample ACE2 binding. Because binding activity of ACE2 to SARS-CoV-2 spike can vary from tissue to tissue and from normal and diseased states, 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-1005-48	96 Assays Cat. #D-1005-96	Storage Upon Receipt
WB (10X Wash Buffer)	14 ml	28 ml	4°C
AB (Assay Buffer)	6 ml	12 ml	RT
AAS (ACE2 Assay Standard, 1 mg/ml)*	5 µl	10 µl	–20°C
ADA (ACE2 Detection Antibody, 1000X)*	6 µl	12 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 **AAS** and **ADA** 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
- ☐ ACE2-Spike protein inhibitors
- ☐ 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 the 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 measuring ACE2 level in various samples is emphasized by the complicated SARS-CoV-2-ACE2 interaction under normal (healthy) and abnormal (pre-existing diseases and viral infection) conditions.

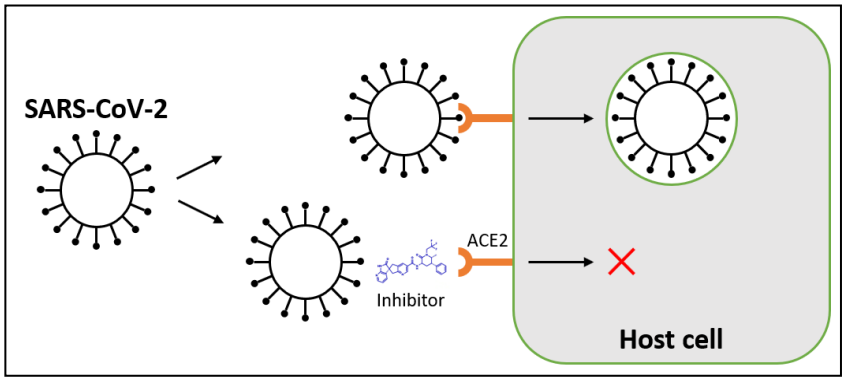

Fig 1. SARS-CoV-2 binding to ACE2 and entry into cells

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ACE2 expression can be measured by RT-PCR or RNA-seq at the RNA level or by western blot and ELISA at the protein level. However, such expression may not reflect the binding affinity or activity of ACE2 to SARS-CoV-2 spike protein, as functional ACE2 would be affected by methylation or acetylation-based post-transcriptional modification [4]. Thus, a SARS-CoV-2-ACE2 binding activity assay would truly reflect functional ACE2 level in binding to SARS-CoV-2. Such an assay will not only detect the ACE2 binding activity under healthy and diseased conditions but also provide a measurement of ACE2 level change under various experimental interventions, including in vivo ACE2 epigenetic regulation, in vivo and in vitro ACE2 activity inhibition (ex: antibody and small molecule inhibitors) and environmental impact (ex: smoking).

There are no/few methods available for detecting SARS-CoV-2-ACE2 binding activity and inhibition with the use of biological samples. To address this issue, EpigenTek developed and offers the CoviDrop™ SARS-CoV-2 Spike-ACE2 Binding Activity/Inhibition Assay 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 45 minutes.
- **Robust:** Innovative kit composition enables background signals to be extremely low and allows the assay to be simple, accurate, reliable, and consistent.
- **Convenient:** Biological samples, including plasma, serum, swab samples, other body fluids, and purified proteins, can be used to detect SARS-CoV-2-ACE2 binding activity/inhibition in vivo and in vitro.
- **Sensitive and Specific:** The activity can be detected from as low as 0.1 ng of ACE2 binding and only specific for ACE2.
- **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.

Reference:

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.

PRINCIPLE & PROCEDURE

The CoviDrop™ SARS-CoV-2 Spike-ACE2 Binding Activity/Inhibition Assay Kit contains all reagents necessary for the measurement 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. ACE2 contained in the sample is captured by the coated spike protein. The amount of the captured ACE2, which is proportional to ACE2 binding activity, is then recognized by anti-ACE2 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.

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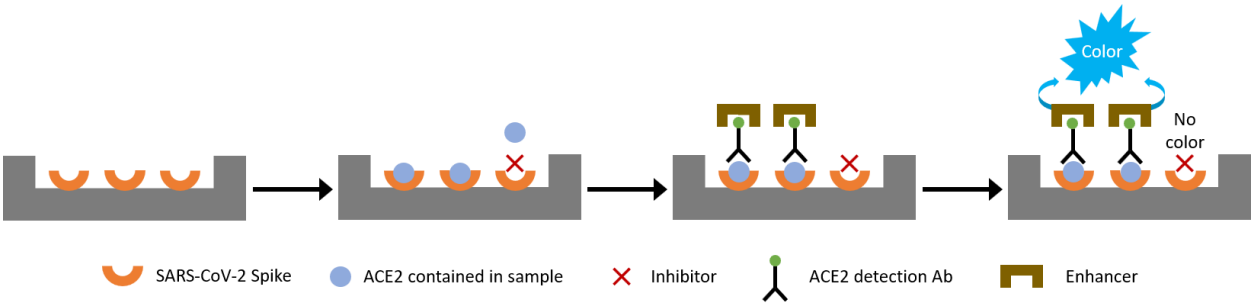

Fig 2. Schematic procedure of the CoviDrop™ SARS-CoV-2 Spike-ACE2 Binding Activity/Inhibition Assay Kit

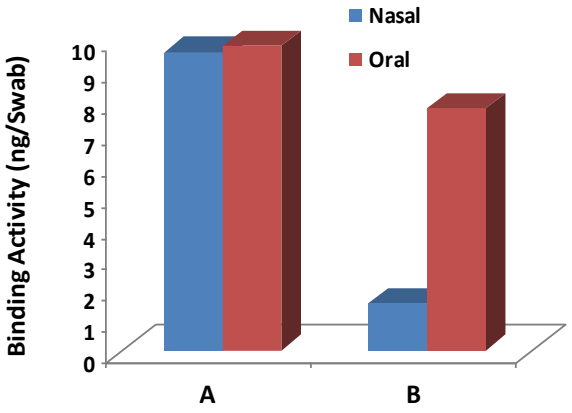

Fig 3. Demonstration of high sensitivity and specificity of the CoviDrop™ SARS-CoV-2 Spike-ACE2 Binding Activity/Inhibition Assay Kit with use of nasal and oral swab samples from different individuals.

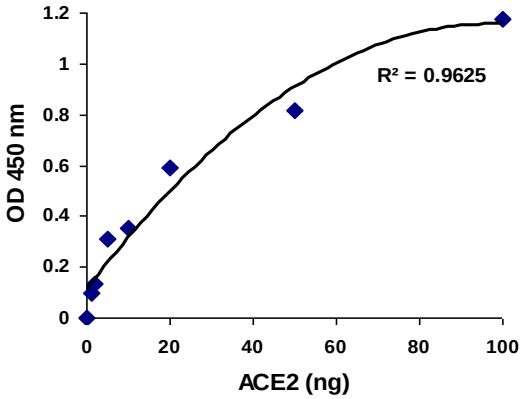

Fig 4. Illustrated dose-response of CoviDrop™ SARS-CoV-2-ACE2 binding assayed with the SARS-CoV-2 Spike-ACE2 Binding Activity/Inhibition Assay Kit.

ASSAY PROTOCOL

Starting Materials

Input Amount: For whole cell/tissue extract, plasma, serum, or body fluid, the sample volume should not be more than 10 µl (10% of total assay solution). For swab samples, the sample can be first released into 300 µl of swab release buffer (#D1005-SAB, EpigenTek) and then used for the assay with 100 µl of the sample solution. For the purified ACE2 protein, the amount can be 0.5 ng to 200 ng, depending on the purity and properties of the protein (wild type or mutated one).

Whole Cell Extraction: You can use your own method of choice for preparing whole cell extracts. EpigenTek also offers a whole cell extraction kit (Cat. No. OP-0003) optimized for use with this kit.

Cell Extract or Purified ACE2 Storage: Cell extract or purified ACE2 should be stored at –80°C until use.

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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 ADA** (ACE2 Detection Antibody) Solution:

Dilute **ADA (**ACE2 Detection Antibody)) with **Diluted WB** (1X Wash Buffer) at a ratio of 1:1000 (i.e., add 1 µl of **ADA** to 1000 µl of **Diluted WB**). About 50 µl of this **Diluted ADA** will be required for each assay well.

c. Prepare **Diluted AAS** (ACE2 Assay Standard):

Suggested Standard Curve Preparation: First, dilute **AAS (**ACE2 Assay Standard) with **AB** (Assay Buffer) to 50 ng/µl by adding 2 µl of **AAS** to 38 µl of **AB**. Then, further prepare six concentrations by combining the 50 ng/µl **Diluted AAS** with **AB** into final concentrations of 0.5, 1, 2, 5, 10, 25, and 50 ng/µl according to the following dilution chart:

Tube	Diluted AAS (50 ng/µl)	AB	Resulting Diluted AAS Concentration
1	1.0 µl	99.0 µl	0.5 ng/µl
2	1.0 µl	49.0 µl	1.0 ng/µl
3	1.0 µl	24.0 µl	2.0 ng/µl
4	1.0 µl	9.0 µl	5.0 ng/µl
5	1.0 µl	4.0 µl	10 ng/µl
6	2.0 µl	2.0 µl	25 ng/µl
7	4.0 µl	0.0 µl	50 ng/µl

***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) For simple comparison of binding activity between the samples, you can only use a single point control, which can be prepared by diluting **AAS** at a concentration of 25 ng/µl.*

2. Binding Reaction

a. Predetermine the number of strip wells required for your experiment. It is advised to run replicate samples (include 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 100 µl of **AB** (Assay Buffer) per well.

c. Positive Control Wells: Add 98 µl of **AB** (Assay Buffer) and 2 µl of **diluted AAS (**ACE2 Assay Standard) at concentrations of 0.5 to 50 ng/µl (see the chart in Step 1c). The final concentrations should be 1, 2, 4, 10, 20, 50 and 100 ng per well.

d. Sample Wells Without Inhibitor or modulator: Add 90 µl to 95 µl of **AB** (Assay Buffer), and 5 µl to 10 µl

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of sample solutions (except swab samples) per well. The total volume should be 100 µl per well.

e. Sample Wells With Inhibitor or modulator: Add 85 µl to 90 µl of **AB** (Assay Buffer), 5 to 10 µl of sample solutions (except swab samples), and 5 µl of inhibitor/modulator solution per well. The total volume should be 100 µl per well.

Note: *(1) Follow suggested well setup diagrams; (2) If using a single point control, add 2 µl of* ***AAS*** *at a concentration of 25 ng/µl, as prepared in Step 1C into the well; (3) For Swab samples released in 300 µl of swab release buffer, add 100 µl of the sample solution for assay. (4) The concentration of inhibitors or modulator 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/modulator can be reduced to 1% of the reaction solution or less.*

f. Tightly cover the strip-well microplate with Parafilm M or aluminium foil to avoid evaporation, and incubate at 37°C for 60 min.

g. 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. Antibody Recognition and Signal Detection

a. Add 50 µl of the **Diluted ADA** (ACE2 Detection Antibody) to each well, then carefully cover with Parafilm M or aluminium foil and incubate at room temperature for 30 min.

b. Remove the **Diluted ADA** (ACE2 Detection Antibody) 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 positive control wells turns medium blue. Mix the solution by gently shaking the frame and wait 1-2 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.*

4. Binding Activity Calculation

For simple calculation:

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

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b. Calculate ACE2 binding activity using the following formula:

$$\textit{Binding Activity (OD/ ml)} = \frac{\textit{(Sample OD – Blank OD)}}{\textit{Sample amount (µl)*}} \times \textit{1000}$$

* Sample volume added into the reaction at step 2d in µl.

Example calculation:

Average OD450 of sample is 0.55
Average OD450 of blank is 0.05
Sample volume is 5 µl

$$\text{Binding activity} = \frac{(0.55 - 0.05)}{5} \times 1000 = 100 \text{ OD/ml}$$

For accurate calculation:

First, generate a standard curve and plot the OD values versus the amount of **AAS** (ACE2 Assay Standard) at each concentration point. Then determine the slope as OD/ng using linear regression (*Microsoft Excel's* linear regression or slope functions are suitable for such calculation) and the most linear part (include at least 4 concentration points) of the standard curve for optimal slope calculation. Now calculate binding activity using the following formula:

$$\text{Binding activity (ng/ml)} = \frac{\text{OD (sample – blank)}}{\text{Slope} \times \text{Sample volume (µl)}} \times 1000$$

When the standard curve is flat due to a saturated signal intensity at high concentration:

a. Graph the second order polynomial curve and obtain second order polynomial regression equation:

$$Y = aX^2 + bX$$

Here, X = Binding activity; Y = <Sample OD> – <Blank OD>; a and b is known Slope 1 and Slope 2, respectively.

* *Microsoft Excel's polynomial regression function could be used for easy and convenient calculation.*

b. Calculate binding activity of the samples based on the following equation, derived from the above equation.

$$\text{Binding activity (ng/ml)} = \frac{(b^2 + 4aY)^{0.5} - b}{-2a} \div S \times 1000$$

Here, **S** is the volume of input sample in µl.

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Note: *(1) For cell/tissue extracts, if the protein concentration is quantified, the binding activity can be expressed as ng/mg protein or OD/mg protein by dividing the measured protein concentration (ex: ng/ml ÷ x mg/ml or OD/ml ÷ x mg/ml); (2) For swab samples, the dilution factor from sample solution should be multiplied by the calculated binding activity to obtain the total binding activity from the swab sample. For example, if 50 µl of sample solution is used for assay and the swab sample is released in 300 µl of swab release buffer, the dilution factor is 6 (300 ÷ 50)*

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 amount 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
AAS (ACE2 Assay Standard)	1 µl	2 µl	2-4 µl
Diluted ADA (ACE2 Detection Antibody)	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	AAS 1 ng	AAS 1 ng	Sample	Sample	Sample	Sample
C	AAS 2 ng	AAS 2 ng	Sample	Sample	Sample	Sample
D	AAS 5 ng	AAS 5 ng	Sample	Sample	Sample	Sample
E	AAS 10 ng	AAS 10 ng	Sample	Sample	Sample	Sample
F	AAS 20 ng	AAS 20 ng	Sample	Sample	Sample	Sample
G	AAS 50 ng	AAS 50 ng	Sample	Sample	Sample	Sample
H	AAS 100 ng	AAS 100 ng	Sample	Sample	Sample	Sample

Note: *For single point control, the **AAS** (ACE2 Assay Standard) at the concentration of 50 ng/well can be added in the strip 1 and strip 2 of B row and all other wells can be used for samples except blank wells.*

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TROUBLESHOOTING

Problem	Possible Cause	Suggestion
No signal or weak signal in both the positive control and 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.
	The well is incorrectly washed before binding reaction.	Ensure the well is not washed prior to adding the standard control and sample.
	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.
No signal or weak signal in only the positive control wells	The assay standard control is insufficiently added to the well in Step 2c.	Ensure a sufficient amount of assay standard control is added. Check if the dilution is properly carried out.
	The quality of the assay standard control has been degraded due to improper storage conditions.	Follow the Shipping & Storage guidance for storage instructions of the assay standard.
High background present in the blank wells	Insufficient washing of wells.	Check if washing recommendations at each step are 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.
No signal or weak signal only in sample wells	Sample is not properly prepared	Ensure the sample preparation is properly carried out. (For example, correct method for whole cell or tissue protein extraction, plasma and serum preparation, etc.)
	Sample volume added into the wells is insufficient.	Ensure a sufficient sample volume is used as indicated in Step 2. The sample can be titrated to determine the optimal amount to use in the assay.
	Sample was not stored properly or has been stored for too long.	Ensure sample is stored at proper temperature.
	Little or no activity of ACE2 contained in the sample.	This problem may be a result of many factors. If the affecting factors cannot be determined, use new or re-prepared samples.
Uneven color development	Insufficient washing of the wells.	Ensure the wells are washed according to the protocol. Ensure any residues from the wash buffer are removed as much as possible.

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	Delayed color development or delayed stopping of color development in the wells.	Ensure color development solution is added sequentially and consistent with the order you added the other reagents (e.g., from well A to well G or from well 1 to well 12).
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.
	Did not use the same pipette device throughout the experiment.	Use the same multi-channel pipette device throughout the entire experiment, as different pipette devices may have slight variations in performance.

RELATED PRODUCTS

Whole Cell Extract Preparation

OP-0003 EpiQuik™ Whole Cell Extraction Kit

SARS-CoV-2 Serological Detection

D-1001 Seroflash™ SARS-CoV-2 IgM/IgG Antibody Detection Kit
D-1002 Seroflash™ SARS-CoV-2 IgM/IgG ELISA Fast Kit
D-1008 SeroFlash™ SARS-CoV-2 Neutralizing Antibody Assay Fast Kit

SARS-CoV-2 Spike-ACE2 Binding Assay

D-1004 CoviDrop™ SARS-CoV-2 Spike-ACE2 Binding Inhibitor Screening Fast Kit
D-1006 CoviDrop™ SARS-CoV-2 Targeted Preprotein Convertase Inhibitor Screening Fast Kit
D-1007 CoviDrop™ SARS-CoV-2 Targeted Proprotein Convertase Activity/Inhibition Assay Kit

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