RayBio® COVID-19 Spike-ACE2 binding assay kit

For COVID-19 drug and antibody screening

User Manual
(Revised Oct. 23rd , 2020)

(Cat#: CoV-SACE2)

RayBiotech, Inc.

ISO 13485 & GLP Certified

Tel: (888) 494-8555
Fax: (770) 206-2393
Web: www.raybiotech.com
Email: info@raybiotech.com

RayBiotech, Inc.

RayBio® COVID-19 Spike-ACE2 binding assay kit protocol

TABLE OF CONTENTS

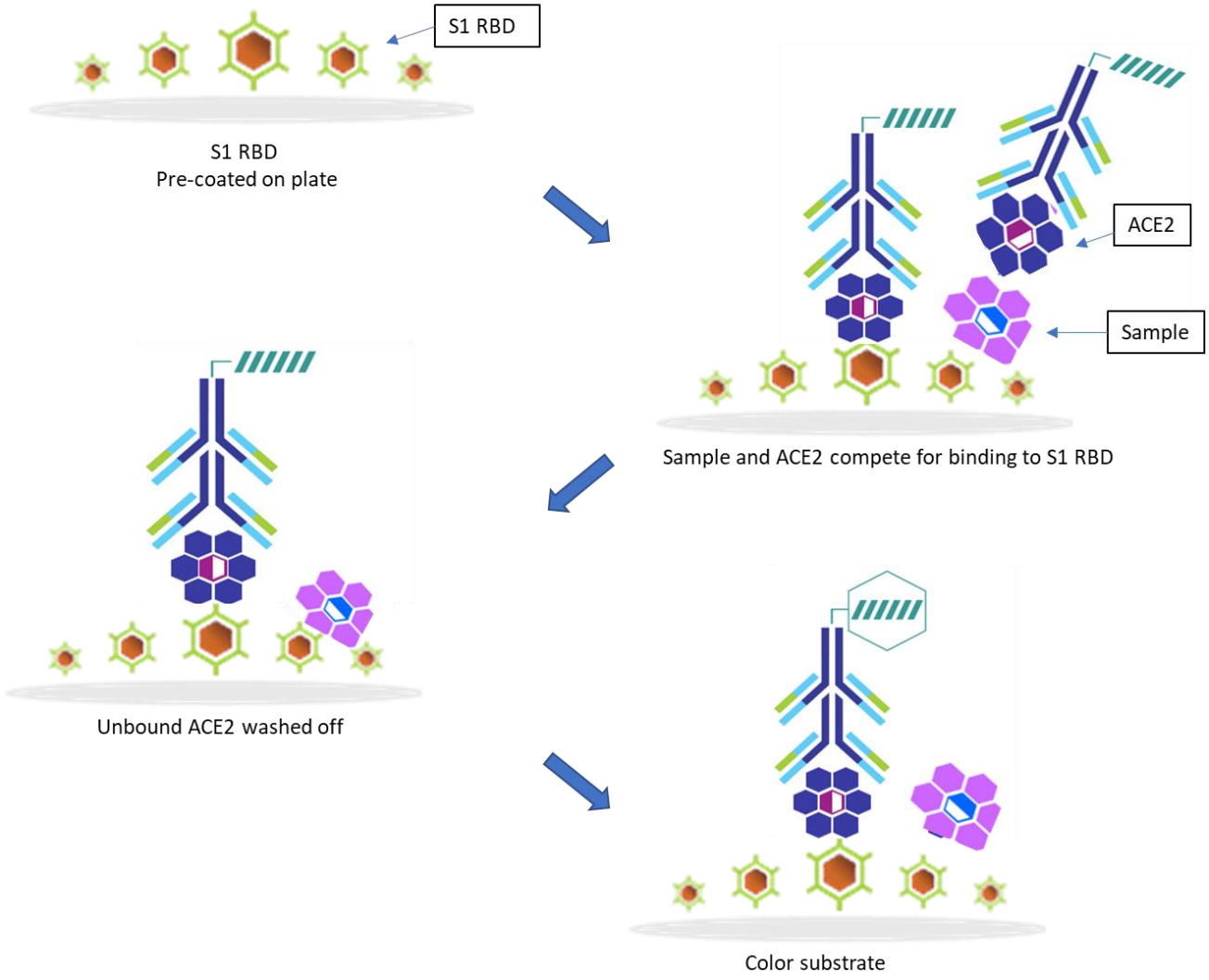
I. INTRODUCTION

The coronavirus disease 2019 (COVID-19) is caused by the SARS-CoV-2 virus. A critical step of infection is when the virus enters human host cells, which is enabled by the interaction between the SARS-CoV-2 Spike (S) protein's receptor binding domain (RBD) on the surface of the viral particle and the Angiotensin I Converting Enzyme 2 (ACE2) receptor on the surface of human cells. Thus, the identification of small molecules, antibodies, or other biological molecules that interfere with the formation of the S-ACE2 complex could help to develop drugs to prevent or treat COVID-19.

The RayBio® COVID-19 Spike-ACE2 binding assay kit is a rapid, simple, and sensitive method to characterize the binding affinity of the S-ACE2 complex in the presence of potential inhibitors. The *in vitro* enzyme-linked immunosorbent assay can measure numerous reagents and conditions simultaneously. For example, this kit can be used for screening inhibitor activity and drugs, vaccine development, and testing potential therapeutic antibodies.

The RayBio® COVID-19 Spike-ACE2 binding assay uses a 96-well plate coated with recombinantly-expressed RBD of the SARS-CoV-2 Spike protein. The testing reagent-of-choice is then added to the wells in the presence of recombinant human ACE2 protein. Unbound ACE2 is removed with washing, and a goat anti-ACE2 antibody is added that binds to the Spike-ACE2 complex. HRP-conjugated anti-goat IgG is then applied to the wells in the presence of 3,3',5,5'-tetramethylbenzidine (TMB) substrate. The HRP-conjugated anti-goat IgG binds to the ACE2 antibody and reacts with the TMB solution, producing a blue color that is proportional to the amount of bound ACE. The HRP-TMB reaction is halted with the addition of the Stop Solution, resulting in a blue-to-yellow color change. The intensity of the yellow color is then measured at 450 nm.

How It Works


S1 RBD
S1 RBD
Pre-coated on plate
ACE2
Sample
Sample and ACE2 compete for binding to S1 RBD
Unbound ACE2 washed off
Color substrate

II. MATERIAL PROVIDED

1. COVID19 S-protein Microplate (Item A): 96 wells (12 strips x 8 wells) coated with recombinant COVID19 S-protein RBD domain.
2. Wash Buffer Concentrate (20x) (Item B): 25 ml of 20x concentrated solution.
3. Assay Diluent (Item E2): 15 ml of 5x concentrated buffer. For diluting testing reagent, ACE2 protein (Item F), detection antibody (Item C) and HRP-conjugated IgG Concentrate (Item D).
4. ACE2 protein (Item F): 2 vials of purified human recombinant ACE2 protein (1 vial is enough to assay half microplate)
5. Detection Antibody ACE2 (Item C-1): 2 vials of goat anti-ACE2 (1 vial is enough to assay half microplate).
6. HRP-conjugated Anti-goat IgG (Item D-1), 15 µl of 1000x concentrated HRP-conjugated anti-goat IgG.
7. TMB One-Step Substrate Reagent (Item H): 12 ml of 3,3',5,5'-tetramethylbenzidine (TMB) in buffered solution.
8. Stop Solution (Item I): 8 ml of 0.2 M sulfuric acid.

III. STORAGE

Upon receipt, the kit should be stored at 4°C. Please use within 6 months from the date of shipment. After initial use, Wash Buffer Concentrate (Item B), Assay Diluent (Item E2), TMB One-Step Substrate Reagent (Item H), Stop Solution (Item I) should be stored at 4°C to avoid repeated freeze-thaw cycles. Return unused wells to the pouch containing a desiccant pack, reseal along entire edge and store at 4°C. Item F, Item C and Item D store at 4°C for up to 6 months to avoid repeated freeze-thaw cycles.

IV. ADDITIONAL MATERIALS REQUIRED

1. Microplate reader capable of measuring absorbance at 450 nm.
2. Shaker.
3. Precision pipettes to deliver 2 µl to 1 ml volumes.
4. Adjustable 1-25 ml pipettes for reagent preparation.
5. 100 ml and 1 liter graduated cylinders.
6. Distilled or deionized water.
7. Tubes to prepare sample dilutions.

V. SAMPLE PREPARATION

Mix testing reagent (e.g., small molecule, antibody) with ACE2 protein concentrate (see Part VI, 4), then dilute the mixture with 1x Assay Diluent dilute to make a 1x ACE2 protein working concentration. Each sample should contain the same 1x ACE2 protein concentration.

Note: It is recommended that all samples be run in at least duplicate. For the initial experiment, we recommend performing a serial dilution (e.g., 5-fold to 5000-fold) to determine the optimal amount of test reagent to use.

EXAMPLE: To test compound A's ability to inhibit Spike-ACE2 binding, dilute the 100 mM stock solution to create a serial solutions of 20 mM, 2 mM, 0.2 mM, 0.02 mM, 0.002 mM and 0 mM in six separate tubes. Pipette 225 µl of 1x ACE2 protein working solution (prepared in VI, 4) into each tube, except the 20 mM (leave this one empty). Pipette 50 µl of compound A stock, 2.5 µl of ACE2 protein concentrate (prepared in VI, 2) and 197.5ul 1x Assay Diluent into the tube labeled 20 mM. Mix thoroughly. Pipette 25 µl of the 20 mM compound A sample into the tube labeled 2 mM. Mix thoroughly. Repeat this step with each successive concentration. For each serial dilution, use 225 µl of 1x ACE2 protein working solution and 25 µl of the prior concentration until the final concentration is reached. Mix each tube thoroughly before the next transfer.

VI. REAGENT PREPARATION

1. Bring all reagents and samples to room temperature (18 - 25°C) before use.

2. 5x Assay Diluent (Item E2) should be diluted 5-fold with deionized or distilled water before use.

3. If the Wash Concentrate (20x) (Item B) contains visible crystals, warm to room temperature and mix gently until dissolved. Dilute 20 ml of Wash Buffer Concentrate into deionized or distilled water to yield 400 ml of 1x Wash Buffer.

4. Briefly spin the ACE2 protein (Item F) before use. Add 50 µl of 1x Assay Diluent into the vial to prepare an ACE2 protein concentrate. Pipette up and down to mix gently (the concentrate can be stored at 4°C for 1-2 days or at -80°C for one month). The ACE2 protein working solution should be diluted 100-fold with 1x Assay Diluent and used in sample preparation.

5. Briefly spin the detection antibody (Item C-1) before use. Add 100 µl of 1x Assay Diluent into the vial to prepare a detection antibody concentrate. Pipette up and down to mix gently (the concentrate can be stored at 4°C for 5 days or at -80°C for one month). The goat anti-ACE2 antibody concentrate should be diluted 55-fold with 1x Assay Diluent and used in step 4 of Part VII Assay Procedure.

6. Briefly spin the HRP-conjugated anti-goat IgG (Item D-1) before use. HRP-conjugated anti-goat IgG concentrate should be diluted 1000-fold with 1x Assay Diluent.

   EXAMPLE: Briefly spin the vial to collect contents to the bottom. Add 5 µl of HRP-conjugated anti-goat IgG concentrate into a tube with 5 mL 1x Assay Diluent, then pipette up and down to mix gently to prepare a 1000-fold diluted HRP-conjugated anti-goat IgG solution. Mix well.

VII. ASSAY PROCEDURE:

1. Bring all reagents to room temperature (18 - 25°C) before use. It is recommended that all samples should be run in at least duplicate.

2. Add 100 µl of each sample into appropriate wells. Cover well with plate holder and incubate for 2.5 hours at room temperature or over night at 4°C with shaking.

3. Discard the solution and wash 4 times with 1x Wash Solution. Wash by filling each well with Wash Buffer (300 µl) using a multi-channel pipette or autowasher. Complete removal of liquid at each step is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against clean paper towels.

4. Add 100 µl of prepared 1x detection antibody, anti-ACE2 (Reagent Preparation step 5) to each well. Incubate for 1 hour at room temperature with shaking.

5. Discard the solution. Repeat the wash as described in Step 3.

6. Add 100 µl of prepared 1x HRP-conjugated anti-goat IgG (see Reagent Preparation Step 6) to each well. Incubate for 1 hour at room temperature with shaking.

7. Discard the solution. Repeat the wash as described in Step 3.

8. Add 100 µl of TMB One-Step Substrate Reagent (Item H) to each well. Incubate for 30 minutes at room temperature in the dark with shaking.

9. Add 50 µl of Stop Solution (Item I) to each well. Read at 450 nm immediately.

VIII. ASSAY PROCEDURE SUMMARY

1. Prepare all reagents and samples as instructed.

⇩

2. Add 100 µl sample to each well.
   Incubate 2.5 hours at room temperature or overnight at 4ºC.

⇩

3. Add 100 µl prepared primary antibody to each well.
   Incubate 1.0 hours at room temperature.

⇩

4. Add 100 µl prepared 1X HRP-Conjugated antibody solution.
   Incubate 1 hour at room temperature.

⇩

5. Add 100 µl TMB One-Step Substrate Reagent to each well.
   Incubate 30 minutes at room temperature.

⇩

6. Add 50 µl Stop Solution to each well.
   Read at 450 nm immediately.

IX. DATA

Data analysis: Determine the average absorbance across the replicate readings per sample.

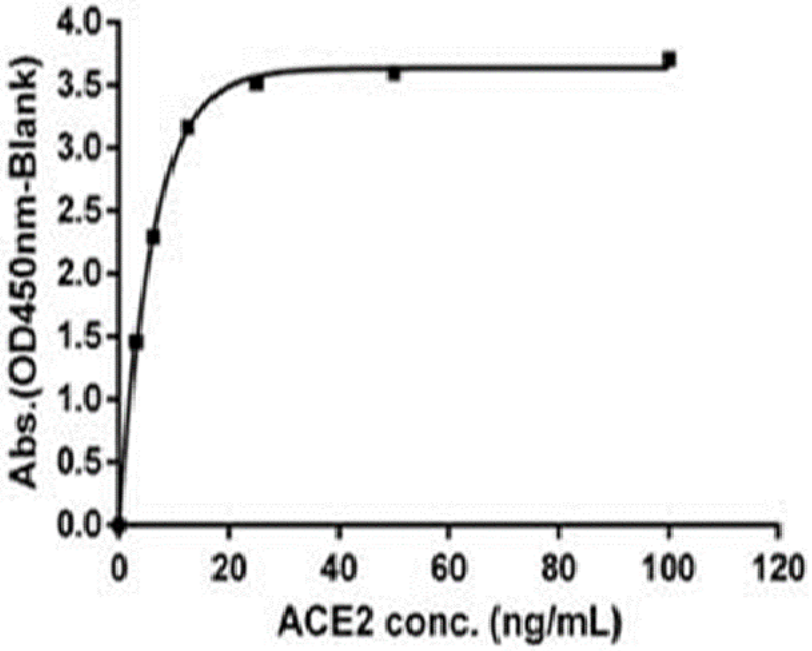


Figure 1. Measurement of serially-diluted human ACE2 protein using the RayBio® COVID-19 Spike-ACE2 binding assay kit

X. TROUBLESHOOTING GUIDE

Problem	Cause	Solution
Too high sample signal	Sample concentration is too low	Increase sample concentration
Large CV	Inaccurate pipetting	Check pipettes
High background	Plate is insufficiently washed	Review the manual for proper washing. If using an automated plate washer, check that all ports are unobstructed.
	Contaminated wash bufer	Make fresh wash buffer
Low "no sample" negative control signal	Improper storage of kit	Upon receipt, the kit should be stored at 4°C
	Stop Solution	Stop solution should be added to each well before measurement and read the OD immediately.
	Improper dilution of primary or secondary antibody	Ensure correct dilution

This product is for research use only.