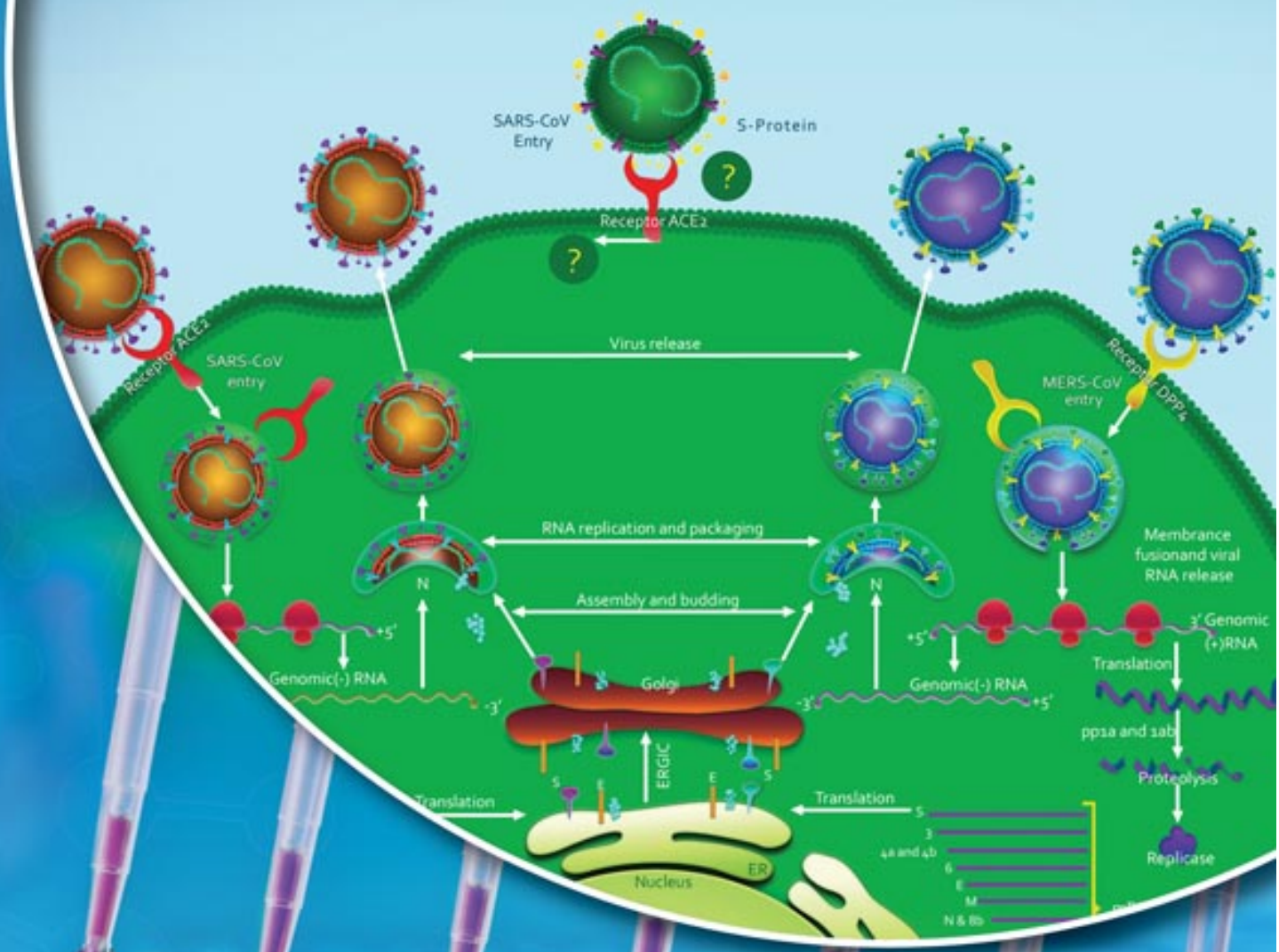
SARS-CoV-2 Inhibitor Screening Kit



Cat. No. 17-3001

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Antibodies & Engineered Cell Lines™

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1. Intended Use

The **SARS-CoV-2 Inhibitor Screening Kit** contains key reagents required to facilitate identification of SARS-CoV-2 inhibitors.

2. Introduction

Coronaviruses (CoVs) are enveloped non-segmented positive-sense single-stranded RNA viruses and can infect respiratory, gastrointestinal, hepatic and central nervous system of human and many other wild animals [1]. Recently, a new severe acute respiratory syndrome β-coronavirus called SARS-CoV-2 (or 2019-nCoV) has emerged, which causes an epidemic of acute respiratory syndrome (called coronavirus human disease 2019 or COVID-19) [2].

SARS-CoV-2 contains 4 structural proteins, including Envelope (E), Membrane (M), Nucleocapsid (N) and Spike (S), which is a transmembrane protein, composed of two subunits S1 and S2 [3]. The S1 subunit contains a receptor binding domain (RBD), which binds to the cell surface receptor Angiotensin-Converting Enzyme 2 (ACE2) present at the surface of epithelial cells, causing mainly infection of human respiratory cells [4].

3. General References

(1) Coronaviridae. Positive Sense RNA Viruses. Available online: https://talk.ictvonline.org/ictv-reports/ictv_9th_report/positive-sense-rna-viruses-2011/w/posrna_viruses/222/coronaviridae

(2) A pneumonia outbreak associated with a new coronavirus of probable bat origin: P. Zhou, et al.; Nature **579,** 270 (2020)

(3) The spike protein of SARS-CoV - a target for vaccine and therapeutic development: L. Du, et al.; Nat. Rev. Microbiol. **7**, 226 (2009)

(4) SARS-CoV-2 Cell Entry Depends on ACE2 and TMPRSS2 and Is Blocked by a Clinically Proven Protease Inhibitor: M. Hoffmann, et al.; Cell **181,** 271 (2020)

4. Assay Principle

This inhibitor screening assay is based on a **colorimetric** ELISA kit, which measures the binding of the RBD of the Spike S protein from SARS-CoV-2 to its human receptor ACE2. Thus, this assay allows identifying and characterizing the effect of different inhibitory molecules including antibodies or chemicals on the inhibition of the binding of SARS-CoV-2 virus to human ACE2.

5. Handling and Storage

- Reagent must be stored at 2-8°C when not in use.
- Plate and reagents should be at room temperature before use.
- Do not expose reagents to temperatures greater than 25°C.

6. Product Specifications

- Number of Assays: Contains sufficient materials for 96 reactions.
- Specificity: Allows inhibitor screening of SARS-CoV-2/ACE2 interaction.
- Stability: Stable at least 1 year after receipt when stored at -20°C.

7. Kit components

• 1 vial ACE2 (human) (rec.) (Biotin) (lyophilized)	10 µg	***(ACE2)***
• 1 vial SARS-CoV-2 Spike S (RBD):Fc (h) (rec.) (lyophilized)	10 µg	**(*SPIKE*)**
• 1 vial HRP Labeled Streptavidin (lyophilized)	2 µg	***(HRP)***
• 1 vial anti-ACE2 (human), mAb (blocking) (AC384) Azide free)	20 µl	**(*INHIB*)**
• 1 vial anti-human IgG (HRP)	120 µl	**(*IgHRP*)**
• 2 bottles Wash Buffer 10x	2 x 30 ml	***(WB 10x)***
• 1 bottle ELISA Buffer 10x	30 ml	***(EB 10x)***
• 1 bottle Blocking Buffer	24 ml	***(BLOCK)***
• 1 bottle TMB K-Blue Aqueous	12 ml	***(TMB)***
• 1 bottle Stop Solution	12 ml	***(STOP)***
• 1 MaxiSorp™flat-bottom 96 well plate	6x16-well strips	
• 2 plate sealers (plastic film)		
• 2 silica gel mini bags		

8. Materials Required

- PBS: 137 mM NaCl, 2.7 mM KCl, 8.1 mM Na_2HPO_4, 1.5 mM KH_2PO_4, pH 7.2, 0.2 µM filtered
- Distilled or deionized water
- Micro titer plate reader at 450nm
- Calibrated precision pipettes. Disposable pipette tips
- Microtubes
- Disposable plastic containers for preparing working buffers
- Plate washer: automated or manual
- Glass or plastic tubes for diluting and aliquoting standard

9. SARS-CoV-2 Spike (RBD)/ACE2-binding Inhibitor Screening Assay

9.1. Reagents Preparation

1. Dilute Wash Buffer 10x ***(WB 10x)*** with deionized water 1:10 before use (e.g. 30 ml Wash Buffer 10X + 270 ml water) to obtain Wash Buffer 1X.

2. Dilute ELISA Buffer 10x ***(EB 10x)*** with deionized water 1:10 before use (e.g. 10 ml ELISA Buffer 10X + 90 ml water) to obtain ELISA Buffer 1X.

3. Reconstitute the SARS-CoV-2 Spike S (RBD):Fc (human) (rec.) **(*SPIKE*)** with 100 µl of deionized water to obtain 0.1 mg/ml. After reconstitution, prepare aliquots and store the reconstituted solution at -20°C. Avoid freeze/thaw cycles. Dilute the reconstituted ***SPIKE*** to the working concentration of 1 µg/ml in PBS and use it fresh.

   *Example: for 1x96-well plate, use 100 µl of **SPIKE** in 10ml PBS*

   Note: Do not use ELISA buffer for protein coating.

4. Reconstitute the ACE2 (human) (rec.) (Biotin) ***(ACE2)*** with 100 µl of deionized water to obtain 0.1 mg /ml. After reconstitution, prepare aliquots and store the reconstituted solution at -20°C. Avoid freeze/thaw cycles.

5. Prepare the Inhibitor Mix Solution (**IMS**) with ACE2 (human) (rec.) (Biotin) **(*ACE2*)** (0.1 mg/ml) to the working concentration of 0.5 µg/ml in ELISA Buffer 1X and use the fresh solution.
   *Example: for 1x96-well plate, use 50 µl of **ACE2** in 10 ml ELISA Buffer 1X*

6. Dilute each SARS-CoV-2 inhibitor (chemicals or antibodies) to be tested (***TEST***) to the desired final concentration in 100 µl of **IMS.**
   Note: Do not exceed 0.1% DMSO if using chemicals as inhibitors

7. For positive inhibitory control, dilute the inhibitory control ACE2 (human), mAb (blocking) (AC384) *(**INHIB**) (*1 mg/ml) to 1 µg/ml in 100 µl of **IMS**.

8. Reconstitute the HRP Labeled Streptavidin **(*HRP*)** with 100 µl of ELISA Buffer 1X.
   Dilute the HRP Labeled Streptavidin **(*HRP*)** to the working concentration by adding 50 µl in 10 ml of ELISA Buffer 1X (1:200).

   After reconstitution, prepare aliquots and store the reconstituted solution at -20°C. Avoid freeze/thaw cycles.

9.2. Assay Procedure

1. Coat the wells by adding 100µl/well of diluted ***SPIKE*** (1 µg/ml) to a 96-well ELISA microplate. Cover the plate with plastic film and leave **overnight (O/N) at 4°C**.

2. Aspirate the coated wells. Remove any remaining liquid by inverting the plate and blotting it against clean absorbent paper.

3. Block plates by adding 200 µl of Blocking Buffer (**BLOCK**) **for 2 hours at room temperature (RT).**

4. Aspirate the coated wells and add 300 µl of Wash Buffer 1X using a multichannel pipette or autowasher. Repeat the process for a total of three washes. After the last wash, remove any remaining Wash Buffer 1X by inverting the plate and blotting it against clean absorbent paper.

5. Add 100 µl/well of **IMS**-diluted SARS-CoV-2 inhibitors to be tested (***TEST***). Cover the plate with plastic film and incubate **for 1 h at 37°C**.
   Note: - We recommend to screen each inhibitor in duplicate
   - Final DMSO concentration in the assay should not exceed 0.1%

6. As a positive control, add 100 µl/well of **IMS**-diluted Inhibitory control ACE2 (human), mAb (blocking) (AC384) ***(INHIB)***. Cover the plate with plastic film and incubate **for 1 h at 37°C**.

7. Repeat the aspiration/wash as in step 4 of Assay Procedure.

8. Add 100 µl/well of the diluted HRP Labeled Streptavidin **(*HRP*)** (1/200). Cover the plate with plastic film and incubate **for 1 hour at RT.**

9. Repeat the aspiration/wash as in step 4 of Assay Procedure.

10. Substrate development is conducted by addition of 100 µl of ready-to-use TMB to each well **for 5 minutes at RT.**

11. Stop the reaction by adding 50 µl of Stop Solution (2M H_2SO_4).

    Note: The Stop Solution (STOP) consists of sulfuric acid. Although diluted, the Stop Solution should be handled with gloves, eye protection and protective clothing

12. Measure the OD at 450 nm using an ELISA reader.

10. Technical Hints and Limitations

- If a negative control is needed to screen chemical inhibitors, dilute the inhibitor solvent (example: DMSO) to 0.1% in 100 µl of **IMS** and test it in duplicates.

- If a negative control is needed to screen inhibitory antibodies, use an unrelated antibody of the same isotype and at the same concentration in 100 µl of **IMS** and test it in duplicates. Once an inhibitor has been identified, calculate the IC_{50} using 2-fold serial dilutions in **IMS** solution starting from the desired concentration.

- If needed, the **SARS-CoV-2 Inhibitor Screening Kit can also be performed in a reverse configuration**, with ACE2 (human) (rec.) (Biotin) **(ACE2)** as coated protein (1 µg/ml) and SARS-CoV-2 Spike S (RBD):Fc (human) (rec.) (**SPIKE**) as binding protein (0.5 µg/ml) present in the Inhibitor Mix Solution **(IMS)**. In this reverse configuration, the anti-human IgG-HRP **(IgHRP)** (1/100 dilution) is needed to detect **SPIKE** binding, instead of the HRP Labeled Streptavidin **(HRP).**

11. Example of Data

Inhibition of *SARS-CoV-2 Spike S (RBD):Fc (human) (rec.)* binding to ACE2 (human) (rec.) (Biotin) by the monoclonal antibody ACE2 (human), mAb (blocking) (AC384) (Azide free) (Cat. No. 29-1005)

The following data are obtained using 2-fold serial dilutions of ACE2 (human), mAb (blocking) (AC384) (Azide free) (***INHIB***) (*in blue)* vs an unrelated control antibody (***Control***) (*in grey)* to inhibit 0.5 µg/ml of ACE2 (h)(rec.) (Biotin) binding to SARS-CoV-2 Spike S (RBD):Fc (human) (rec.) protein. The IC50 is about 450 ng/ml (≅3nM).

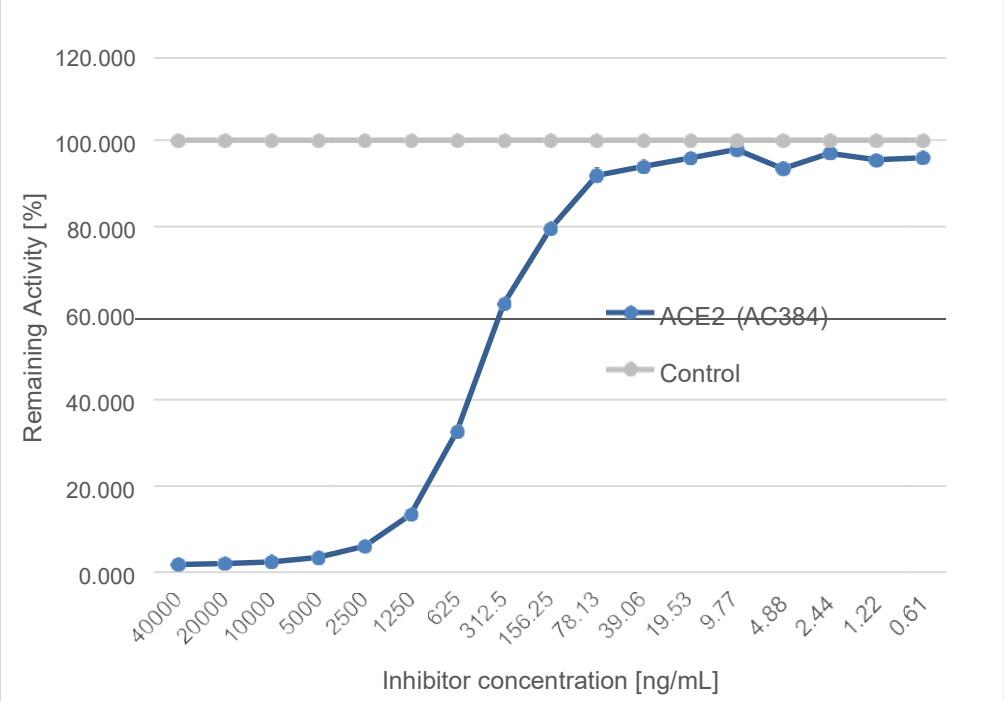
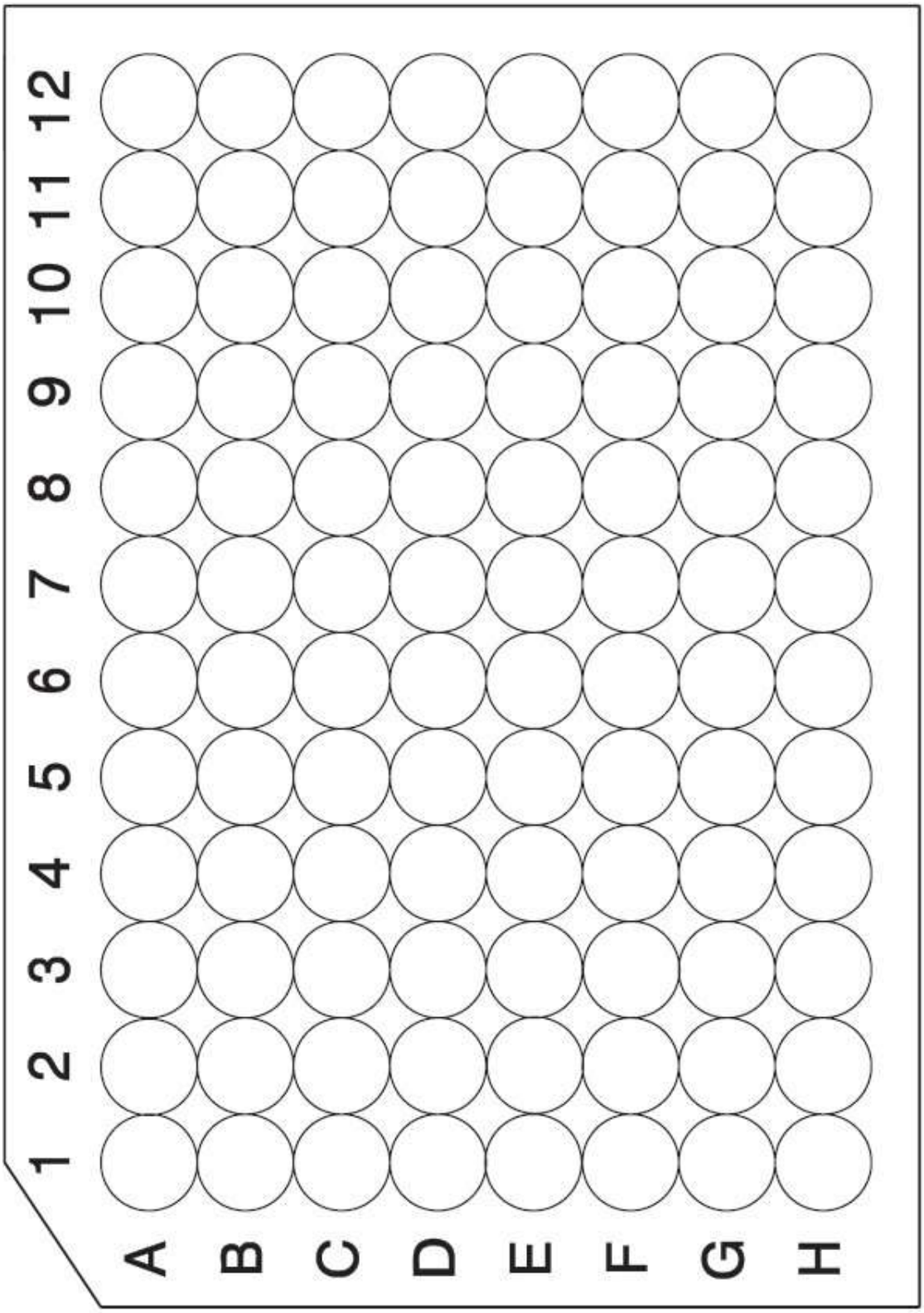
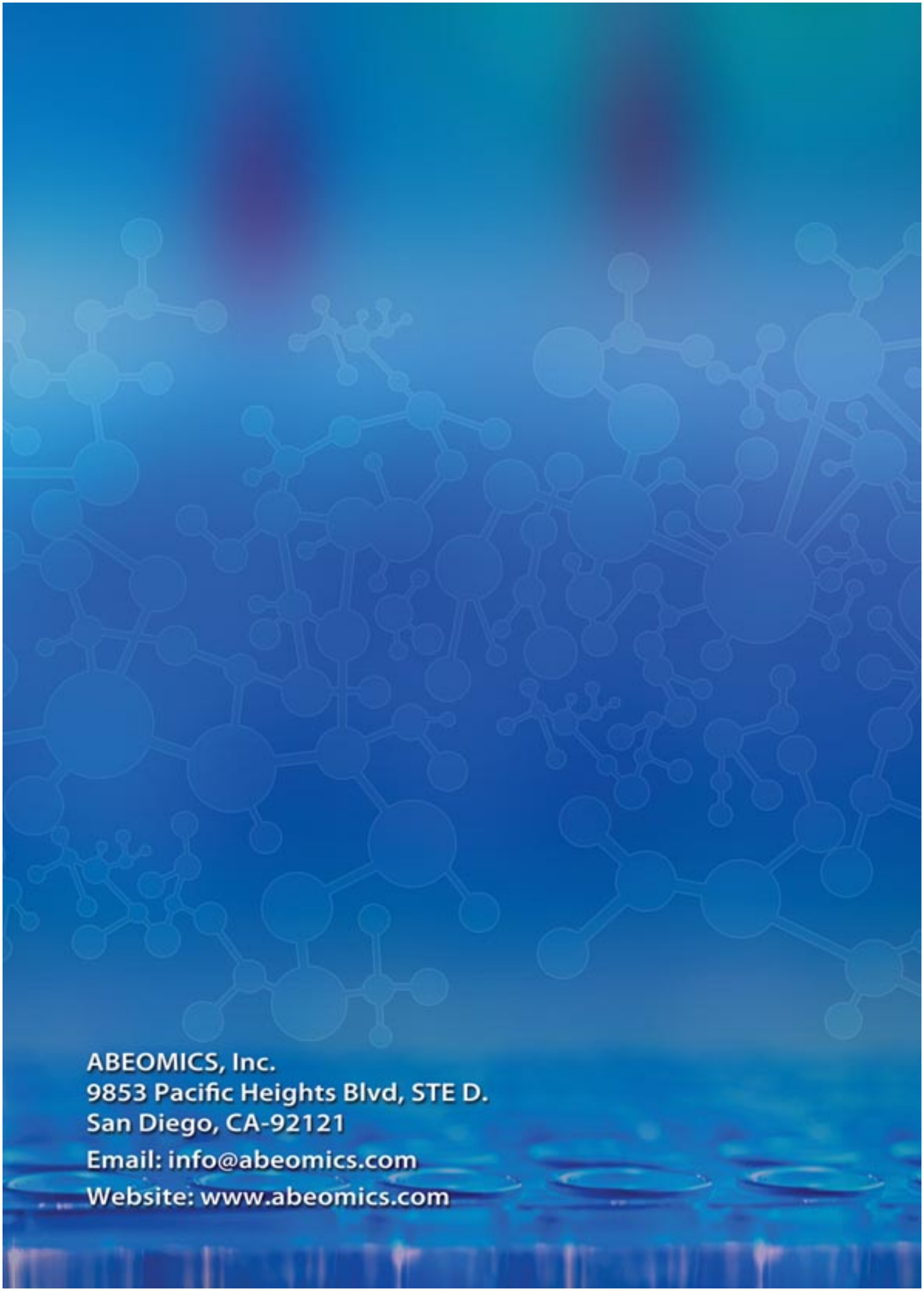


Figure: Binding of human ACE2 to the Spike protein of SARS-CoV-2 is inhibited by anti-ACE2 (human), mAb (blocking) (AC384) Azide free (Cat No# 29-1005).

Plate Layout Template

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