Human SARS-CoV-2 Spike Protein Inhibitor Screening Kit

For detection and measurement of compounds that inhibit binding of viral SARS-CoV-2 Spike protein to the human ACE2 receptor

Catalog #100-0700 1 Kit

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Product Description

The Human SARS-CoV-2 Spike Protein Inhibitor Screening Kit is designed for the identification and characterization of inhibitor molecules, including antibodies or chemicals, that prevent the binding of SARS-CoV-2 virus to the host angiotensin-converting enzyme 2 (ACE2)-expressing cells.

The Spike (S) protein is a type I transmembrane glycoprotein present on the surface of coronaviruses (CoVs). Entry of CoV, including SARS-CoV-2, into host cells is mediated by the S protein, where it interacts with the cell-surface receptor ACE2. In humans, ACE2 is expressed in several organs and tissues, including the intestinal and respiratory epithelia. The S protein has been shown to play a key role in the induction of neutralizing antibody and T cell responses, which may lead to protective immunity.

This inhibitor screening assay is based on a competitive ELISA method, in which inhibitors to be tested are mixed with biotinylated recombinant human ACE2 and are added to ELISA strip plates coated with recombinant SARS-CoV-2 S protein. The captured biotinylated ACE2 is detected by streptavidin-horseradish peroxidase (SA-HRP), which binds the biotinylated ACE2. Addition of the chromogenic enzyme substrate 3,3',5,5' tetramethylbenzidine (TMB) results in a colored product with an intensity directly proportional to the binding activity of ACE2 to the S protein. Inhibitors will compete with the biotinylated ACE2 for binding to the SARS-CoV-2 S protein, resulting in reduced signal for more effective inhibitors.

This inhibitor screening assay can be performed in the reverse configuration, in which the plate is coated with biotinylated recombinant ACE2, recombinant S Protein is mixed with the inhibitor to be tested, and S protein binding is detected using anti-human IgG-HRP.

Product Information

All components listed below are stable until expiry date (EXP) on label. Kit may be shipped at room temperature (15 - 25°C) but should be stored at 2 - 8°C upon receipt. Once components are opened, store at 2 - 8°C and use within 1 month.

COMPONENT NAME	COMPONENT #	SIZE	STORAGE	DESCRIPTION
SARS-CoV-2 Inhibitor Screening Plate	300-0310	1 plate	Store at 2 - 8°C.	Flat-bottom 96-well plate (6 strips x 16 wells)
Biotinylated Human ACE2	300-0305	10 µg	Store at 2 - 8°C.	Lyophilized biotinylated recombinant human ACE2
SARS-CoV-2 Spike Protein (RBD)	300-0306	10 µg	Store at 2 - 8°C.	Lyophilized recombinant SARS-CoV-2 Spike protein (receptor-binding domain [RBD]) with human Fc tag
Human IgG HRP-Conjugated Detection Antibody	300-0309	120 µL	Store at 2 - 8°C.	Horseradish peroxidase (HRP)-conjugated anti-human IgG antibody (100X)
Human ACE2 Antibody Positive Control	300-0308	20 µL	Store at 2 - 8°C.	Anti-human ACE2 blocking antibody, clone AC384
Adhesive Plate Covers	300-0316	2 covers	Store at 2 - 8°C.	For covering plates during incubation
Stop Solution*	300-0315	12 mL	Store at 2 - 8°C.	2 M Sulfuric acid
TMB Substrate	300-0314	12 mL	Store at 2 - 8°C.	3,3',5,5' tetramethylbenzidine (TMB) enzyme substrate solution
ELISA Buffer (10X)	300-0312	30 mL	Store at 2 - 8°C.	Concentrated buffer solution for diluting reagents and samples
Wash Buffer (10X)	300-0311	2 x 30 mL	Store at 2 - 8°C.	Concentrated buffer solution for washing plates
SA-HRP	300-0307	2 µg	Store at 2 - 8°C.	Streptavidin-horseradish peroxidase (SA-HRP) conjugate
Blocking Buffer	300-0313	24 mL	Store at 2 - 8°C.	For blocking plates

*Please refer to the Safety Data Sheet (SDS) for hazard information.

Materials Required But Not Included

- Biohazard safety cabinet certified for Level II handling of biological materials
- Microplate reader set at a wavelength of 450 nm
- ELISA plate washer: Automatic (adaptable for ELISA strip plates) or manual (e.g. multi-pipette or squirt bottle)
- Pipettor with appropriate tips
- Beakers, flasks, and graduated cylinders necessary for reagent preparations
- Tubes for sample dilutions
- Timer
- Absorbent paper
- Deionized or distilled water
- Phosphate-buffered saline (PBS)

Preparation of Reagents and Materials

NOTE: Use clean or disposable glass or plasticware for preparation and storage of reagents and materials.

A. Wash Buffer

Dilute Wash Buffer (10X) 1 in 10 with distilled or deionized water.

Example: Prepare 300 mL wash buffer (1X) by adding 30 mL Wash Buffer (10X) to 270 mL distilled or deionized water.

B. ELISA Buffer

Dilute ELISA Buffer (10X) 1 in 10 with deionized water.

Example: Add 10 mL ELISA Buffer (10X) to 90 mL distilled or deionized water.

C. ELISA Plate

1. Allow plate to adjust to room temperature (15 - 25°C) before opening the bags.
2. Plan the experiment to include a positive control, background control, blank, and test sample wells.
3. Assemble the required number of strips in the plate frame. Store the remaining strips in the foil bag containing the desiccant at 2 - 8°C.

STANDARD AND REVERSE ASSAY CONFIGURATIONS

The assay may be performed in standard or reverse configuration, as follows:

- **Standard**: SARS-CoV-2 Spike Protein (RBD) is the coated protein, Biotinylated Human ACE2 is the binding protein, and SA-HRP is used for detection.
- **Reverse**: Biotinylated Human ACE2 is the coated protein, SARS-CoV-2 Spike Protein (RBD) is the binding protein, and Human IgG HRP-Conjugated Detection Antibody is used for detection.

Prepare reagents for either standard configuration (I) OR reverse configuration (II) as described below.

I. STANDARD CONFIGURATION

D. SARS-CoV-2 Spike Protein (RBD)

1. Add 100 µL distilled or deionized water to the vial of SARS-CoV-2 Spike Protein (RBD) (final concentration 0.1 mg/mL).
   NOTE: If not used immediately, aliquot and store at -20°C. Avoid repeated freeze-thaw cycles.
2. Dilute reconstituted SARS-CoV-2 Spike Protein (RBD) 1 in 100 in PBS (final concentration 1 µg/mL). Mix thoroughly.
   Example: Add 100 µL reconstituted SARS-CoV-2 Spike Protein (RBD) to 10 mL PBS.

E. Biotinylated Human ACE2

Add 100 µL distilled or deionized water to the vial of Biotinylated Human ACE2 (final concentration 0.1 mg/mL). Mix thoroughly.

NOTE: If not used immediately, aliquot and store at -20°C. Avoid repeated freeze-thaw cycles.

F. Inhibitor Mix Solution (IMS): Biotinylated Human ACE2

To prepare the Inhibitor Mix Solution (IMS), dilute reconstituted Biotinylated Human ACE2 (prepared in section E) 1 in 200 in ELISA buffer (prepared in section B) (final concentration 0.5 µg/mL). Mix thoroughly. Use immediately.

Example: Add 50 µL reconstituted Biotinylated Human ACE2 to 10 mL ELISA buffer (1X).

G. SARS-CoV-2 Inhibitor Samples

Dilute each SARS-CoV-2 inhibitor sample (chemicals or antibodies) to be tested to the desired final concentration in 100 µL IMS (prepared in section F).

NOTE: If using chemicals as inhibitors, do not exceed 0.1% dimethyl sulfoxide (DMSO).

H. ACE2 Blocking Antibody (Positive Control)

Dilute Human ACE2 Blocking Antibody 1 in 100 in IMS (prepared in section F). Mix thoroughly.

Example: Add 1 µL Human ACE2 Blocking Antibody to 100 µL IMS.

I. SA-HRP

1. Add 100 µL ELISA buffer (prepared in section B) to the vial of SA-HRP (final concentration 20 µg/mL).
2. Dilute reconstituted SA-HRP 1 in 200 in ELISA buffer (final concentration 0.1 µg/mL). Mix thoroughly.

   Example: Add 50 µL reconstituted SA-HRP to 10 mL ELISA buffer (1X).

   NOTE: If not used immediately, aliquot and store at -20°C. Avoid repeated freeze-thaw cycles.

II. REVERSE CONFIGURATION

J. Biotinylated Human ACE2

1. Add 100 µL distilled or deionized water to the vial of Biotinylated Human ACE2 (final concentration 0.1 mg/mL). Mix thoroughly.

   NOTE: If not used immediately, aliquot and store at -20°C. Avoid repeated freeze-thaw cycles.
2. Dilute reconstituted Biotinylated Human ACE2 1 in 100 in PBS (final concentration 1 µg/mL). Mix thoroughly.

K. SARS-CoV-2 Spike Protein (RBD)

Add 100 µL distilled or deionized water to the vial of SARS-CoV-2 Spike Protein (RBD) (final concentration 0.1 mg/mL).

NOTE: If not used immediately, aliquot and store at -20°C. Avoid repeated freeze-thaw cycles.

L. Inhibitor Mix Solution (IMS): SARS-CoV-2 Spike Protein (RBD)

Dilute reconstituted SARS-CoV-2 Spike Protein (RBD) (prepared in section K) 1 in 200 in ELISA buffer (prepared in section B) (final concentration 0.5 µg/mL). Mix thoroughly. Use immediately.

Example: Add 50 µL reconstituted SARS-CoV-2 Spike Protein (RBD) to 10 mL ELISA buffer (1X).

M. SARS-CoV-2 Inhibitor Samples

Dilute each SARS-CoV-2 inhibitor sample (chemicals or antibodies) to be tested to the desired final concentration in 100 µL IMS (prepared in section L).

NOTE: If using chemicals as inhibitors, do not exceed 0.1% dimethyl sulfoxide (DMSO).

N. ACE2 Blocking Antibody (Positive Control)

Dilute Human ACE2 Blocking Antibody 1 in 100 in IMS (prepared in section L). Mix thoroughly.

Example: Add 1 µL Human ACE2 Blocking Antibody to 100 µL IMS.

O. Human IgG HRP Detection Antibody

Dilute Human IgG HRP-Conjugated Detection Antibody 1 in 100 in ELISA buffer (prepared in section B). Mix thoroughly.

Example: Add 100 µL Human IgG HRP-Conjugated Detection Antibody to 10 mL ELISA buffer (1X).

Directions for Use

Please read the entire protocol before proceeding.

NOTE: Cross-contamination of reagents may invalidate assay results. Permanently labeled, dedicated, multi-channel micropipette reservoirs for reagents are recommended.

NOTE: In all washing steps, each well must be thoroughly washed by filling with wash buffer, then blotted dry by tapping the plate upside down on absorbent paper. Bring all reagents and samples to room temperature (15 - 25°C).

1. Prepare wash buffer (1X), ELISA buffer (1X), ELISA plate, and reagents/samples for either the standard or reverse assay configuration as described in the Preparation section.
2. Standard configuration: Add 100 µL/well of SARS-CoV-2 Spike Protein (RBD) (Preparation section D) to coat the wells.

   OR

   Reverse configuration: Add 100 µL/well of Biotinylated Human ACE2 (Preparation section J) to coat the wells.
3. Cover the plate with an Adhesive Plate Cover and leave at 2 - 8°C overnight.

4. Aspirate the coated wells. Remove any remaining liquid by inverting the plate. Blot dry by tapping the plate upside down on absorbent paper.
5. Add 200 µL/well of Blocking Buffer and incubate at room temperature for 2 hours.
6. Aspirate the coated wells. Wash each well with 3 x 300 µL of wash buffer and blot dry.
7. Add 100 µL/well of IMS-diluted SARS-CoV-2 inhibitor samples to be tested (prepared in section G [standard config.] or section M [reverse config.]).

   NOTE: Duplicate wells for all samples are recommended.
8. As a positive control, add 100 µL/well of ACE2 Blocking Antibody (prepared in section H [standard config.] or section N [reverse config.]).
9. Cover the plate with an Adhesive Plate Cover and incubate at 37°C for 1 hour.
10. Aspirate wells. Wash each well with 3 x 300 µL of wash buffer and blot dry.
11. Standard configuration: Add 100 µL/well of diluted SA-HRP (Preparation section I). Leave blank wells empty.

    OR

    Reverse configuration: Add 100 µL/well of Human IgG HRP Detection Antibody (Preparation section O). Leave blank wells empty.
12. Cover the plate with an Adhesive Plate Cover and incubate at room temperature for 1 hour.
13. Aspirate wells. Wash each well with 3 x 300 µL of wash buffer and blot dry.
14. Add 100 µL of TMB Substrate to all wells (including blank wells).

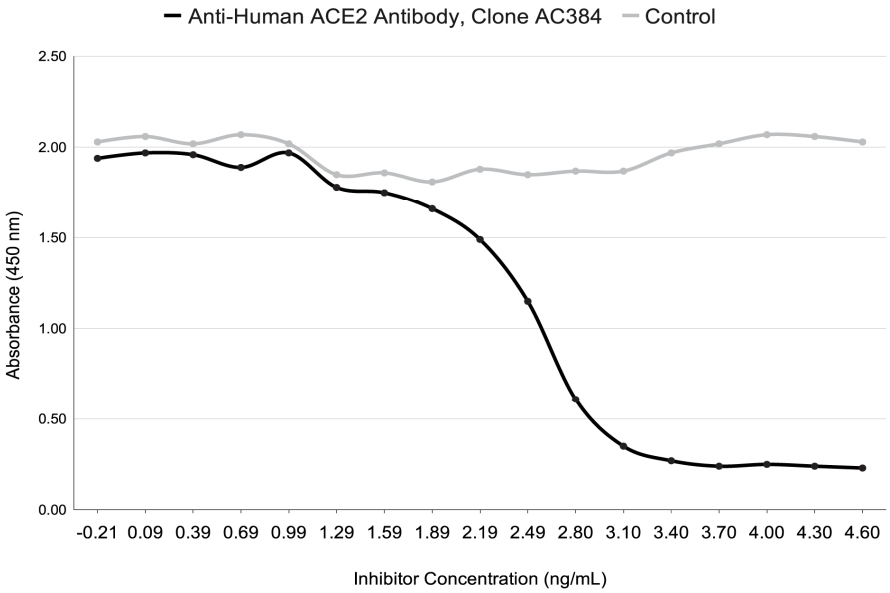
    NOTE: TMB Substrate should be colorless or light blue until added to the plate. Protect TMB Substrate from light.
15. Incubate at room temperature for 5 minutes.
16. Add 50 µL Stop Solution to all wells (including blank wells), in the same order as TMB Substrate addition. Gently tap the plate to ensure thorough mixing.

    NOTE: Use care when handling Stop Solution. Please refer to the Safety Data Sheet (SDS) for hazard information.

    NOTE: The color in the wells will turn from blue to yellow after addition of Stop Solution. Wells that are green indicate they are not thoroughly mixed.
17. Measure the absorbance at 450 nm in a microplate reader. Blank the reader using ELISA wells containing TMB Substrate and Stop Solution only.

    NOTE: Before measuring absorbance, remove any air bubbles in the wells using a small hypodermic needle or a pipette tip.
18. Calculate the average absorbance of each sample from the duplicate values. Subtract the mean absorbance value of the blank from the background control and the sample values.

Data



Binding of biotinylated human ACE2 to the Spike protein of SARS-CoV-2 coated on the ELISA plate is inhibited by the anti-human ACE2 antibody (clone AC384) positive control (black line). The gray line represents a non-inhibitory negative control.

Troubleshooting

PROBLEM	POSSIBLE CAUSE	RECOMMENDED ACTION
Absorbance values too low	Incubation time too short	Follow the directions for the multiple incubation steps outlined in Directions for Use.
	Undiluted Wash Buffer (10X)	Dilute Wash Buffer (10X) with distilled or deionized water as described in Preparation of Reagents and Materials, section A.
	Incorrect dilution of SA-HRP or Detection Antibody	Follow the directions for dilution of SA-HRP or Detection Antibody in the Preparation section.
	Wash step omitted before adding SA-HRP or Detection Antibody	Follow sequence of incubation and wash steps as outlined in Directions for Use.
Absorbance values too high	Incubation time too long	Follow the directions for the multiple incubation steps outlined in Directions for Use.
	Insufficient washing	Ensure that each well is washed 3X with 300 µL of diluted Wash Buffer. Ensure that the multi-channel pipette fills and empties reproducibly. Blot wells dry before proceeding to the next step. If using an automated plate washer, add a 30-second soak period following the addition of wash buffer, and/or rotate the plate 180 degrees between wash steps.
	Incorrect dilution of SA-HRP or Detection Antibody	Follow the directions for dilution of SA-HRP or Detection Antibody in the Preparation section.
Low absorbance readings despite good color development in wells	Incorrect wavelength setting on the ELISA reader	Check that the measuring wavelength is set at 450 nm and that the ELISA reader has the correct filter for this wavelength.
High background	Insufficient washing	Ensure that each well is washed 3X with 300 µL of diluted Wash Buffer. Ensure that the multi-channel pipette fills and empties reproducibly. Blot wells dry before proceeding to the next step.
	Incorrect dilution of SA-HRP or Detection Antibody	Follow the directions for dilution of SA-HRP or Detection Antibody in the Preparation section.
Poor replicates	Insufficient washing	Ensure that each well is washed 3X with 300 µL of diluted Wash Buffer. Ensure that the multi-channel pipette fills and empties reproducibly. Blot wells dry before proceeding to the next step. If using an automated plate washer, add a 30-second soak period following the addition of wash buffer, and/or rotate the plate 180 degrees between wash steps.
	Unequal volumes in wells	Ensure that pipettes function properly.
	Carry-over between wells; evaporation from wells; splashing of well contents onto adhesive cover	Always use a new Adhesive Plate Cover for each incubation. Ensure that each well is sealed tightly. Keep filled plates in a horizontal position and handle with caution.
	Samples not mixed after thawing	Vortex samples after thawing.
	High concentration of lipids or particulate matter in samples	Filter or centrifuge samples to pellet aggregates. Lipids may concentrate on the surface after centrifugation and may be removed.
	Air bubbles in well during measurement with the ELISA reader	Remove air bubbles using a small hypodermic needle or pipette tip.

Related Products

For a complete list of ELISA kits and related products from STEMCELL Technologies, visit www.stemcell.com or contact us at techsupport@stemcell.com.