CREATIVE BIOMART®
Assay Kit

ACE2: Spike RBD (SARS-CoV-2) Inhibitor Screening Assay Kit

Product Information

Cat.No. Kit-5201

Product Overview

The ACE2:SARS-CoV-2 Spike Inhibitor Screening Assay Kit is designed for screening and profiling inhibitors of this interaction. This kit comes in a convenient 96-well format, with purified ACE2 and SARS-CoV-2 Spike proteins, HRP-labeled anti-mouse Fc region antibody, and assay buffer for 100 binding reactions. The key to this kit is the high sensitivity of detection of Fc-tagged Spike protein by HRP-labeled Anti-mouse-Fc. Only a few simple steps on a microtiter plate are required for the assay. First, ACE2 protein is attached to a nickel-coated 96-well plate. Next, SARS-CoV-2 Spike-Fc is incubated with ACE2 on the plate. Finally, the plate is treated with Antimouse-Fc-HRP followed by addition of an HRP substrate to produce chemiluminescence, which then can be measured using a chemiluminescence reader.

Description

Coronavirus disease 2019 (COVID-19) increases the risk of developing Acute Respiratory Distress Syndrome (ARDS), which is often fatal at the late stages of the infection when the SAR-CoV-2 virus causes significant damage to the lungs. As a first step of the viral replication strategy, the virus attaches to the host cell surface before entering the cell. The Spike protein receptor binding domain (RBD) recognizes and attaches to the Angiotensin-Converting Enzyme 2 (ACE2) receptor found on the surface of type I and II pneumocytes, endothelial cells, and ciliated bronchial epithelial cells. Drugs targeting the interaction between the Spike protein of SARS-CoV-2 and ACE2 may offer some protection against the viral infection.

Stability

Up to 6 months from date of receipt, when stored as recommended.

Size

96 reactions

Kit Components

SARS-CoV-2 Spike Protein (RBD), mFc Tag, 5 μg, -80°C

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ACE2, His-Tag, 5 µg, -80°C
3x Immuno Buffer 1, 50 ml, -20°C
Blocking Buffer 2, 50 ml, +4°C
Secondary HRP-labeled antibody 1 (mouse), 15 µl, -80°C
ELISA ECL substrate A (transparent bottle), 6 ml, Room Temp
ELISA ECL substrate B (brown bottle), 6 ml, Room Temp
Nickel-coated 96-well white microplate, 1, +4°C
Avoid multiple freeze/thaw cycles!

Materials Required but Not Supplied

PBS (Phosphate buffered saline)
Luminometer or fluorescent microplate reader capable of reading chemiluminescence
Adjustable micropipettor and sterile tips

Technical Notes

This kit is based on binding to the mouse Fc region. If your sample includes IgG, it may interfere with the assay and create a false positive signal.

Assay Protocol

All samples and controls should be tested in duplicate.
Coating the plate with ACE2-His:
1) Thaw ACE2-His on ice. Upon first thaw, briefly spin tube containing ACE2-His to recover the full contents of the tube. Aliquot into single use aliquots. Immediately store remaining ACE2-His in aliquots at -80°C. Note: ACE2-His is very sensitive to freeze/thaw cycles. Avoid multiple freeze/thaw cycles.
2) Dilute ACE2-His to 1 µg/ml in PBS.
3) Add 50 µl of diluted ACE2-His solution to each well and incubate at room temperature for one hour with slow shaking.
4) Dilute 3x Immuno Buffer 1 to 1x Immuno Buffer 1 with water. Dilute only the amount required for the assay; store remaining 3x Immuno Buffer 1 undiluted.
5) Decant to remove supernatant. Wash the plate three times with 100 µl 1x Immuno Buffer 1. Tap plate onto clean paper towels to remove liquid. Block wells by adding 100 µl of Blocking Buffer 2 to

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each well. Incubate for 10 minutes at room temperature with slow shaking. Remove supernatant as described in step 5.

Step 1:
1) Add 20 µl of 1x Immuno Buffer 1 to each well.
2) Add 10 µl of inhibitor solution to each well designated "Test Inhibitor." For the "Positive Control" and "Blank," add 10 µl of the same solution without inhibitor (inhibitor buffer). Optionally, incubate at room temperature for one hour with slow shaking.
Note: It is recommendable to use PBS to dilute antibodies or other proteins acting as neutralization inhibitors. When using small molecules dissolved in DMSO, final DMSO concentration in the assay should be ≤1%. Inhibitor buffer should contain the same concentration of DMSO as the test inhibitor.
3) Thaw SARS-CoV-2 Spike on ice. Upon first thaw, briefly spin tube containing enzyme to recover full contents of the tube. Aliquot SARS-CoV-2 Spike into single use aliquots. Immediately store remaining undiluted enzyme in aliquots at -80°C. Note: SARS-CoV-2 Spike is very sensitive to freeze/thaw cycles. Do not re-use thawed aliquots or diluted enzyme.
4) Dilute SARS-CoV-2 Spike to 1 ng/µl (approximately 20 nM) in 1x Immuno Buffer 1. Keep diluted protein on ice until use. Discard any unused diluted protein after use.
5) Add another 20 µl of 1x Immuno Buffer 1 to the wells designated "Blank".

	Blank	Positive Control	Test Inhibitor
1x Immuno Buffer 1	40 µl	20 µl	20 µl
Test Inhibitor	-	-	10 µl
5% DMSO in water (Inhibitor buffer)	10 µl	10 µl	-
SARS-CoV-2 Spike (1 ng/µl)	-	20 µl	20 µl
Total	50 µl	50 µl	50 µl

6) Initiate reaction by adding 20 µl of diluted SARS-CoV-2 Spike (see Step 1-4) to wells labeled "Positive Control" and "Test Inhibitor". Incubate at room temperature for one hour with slow shaking.
7) Decant to remove supernatant. Wash the plate 3 times with 100 µl/well 1x Immuno Buffer 1. Tap plate onto clean paper towels to remove liquid.
8) Block wells by adding 100 µl of Blocking Buffer 2 to each well. Incubate for 10 minutes at room temperature. Remove supernatant as in Step 1-7.

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Step 2:
1) Dilute Secondary HRP-labeled antibody 1 1000-fold with Blocking Buffer 2.
2) Add 100 µl to each well. Incubate for 1 hour at room temperature with slow shaking.
3) Wash plate three times with 1x Immuno Buffer 1. Tap plate onto clean paper towel to remove liquid.
4) Block wells by adding 100 µl of Blocking Buffer 2 to each well. Incubate for 10 minutes at room temperature. Decant to remove supernatant. Tap plate onto clean paper towels to remove liquid.
5) Just before use, mix 50 µl ELISA ECL Substrate A and 50 µl ELISA ECL Substrate B, then add 100 µl to each well. Discard any unused chemiluminescent reagent after use.
6) Immediately read sample in a luminometer or microtiter-plate capable of reading chemiluminescence. "Blank" value is subtracted from all readings.

Reading Chemiluminescence:
Chemiluminescence is the emission of light (luminescence) which results from a chemical reaction. The detection of chemiluminescence requires no wavelength selection because the method used is emission photometry and is not emission spectrophotometry. To properly read chemiluminescence, make sure the plate reader is set for LUMINESCENCE mode. Typical integration time is 1 second; delay after plate movement is 100 milliseconds. Do not use a filter when measuring light emission. Typical settings for the Synergy 2 BioTek plate reader are: use the "hole" position on the filter wheel; Optics position: Top; Read type: endpoint. Sensitivity may be adjusted based on the luminescence of a control assay without enzyme (typically we set this value as 100).