Product Manual

SARS-CoV-2 Spike Protein S1 ELISA Kit

Catalog Number

VPK-5155 96 assays

VPK-5155-5 5 x 96 assays

FOR RESEARCH USE ONLY Not for use in diagnostic procedures



Introduction

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is a novel form of coronavirus. It was first identified in three people related to the cluster of acute respiratory illness cases in Wuhan, China. SARS-CoV-2 can have effects on the sinuses, nose, and throat (upper respiratory tract) as well as the lungs and windpipe (lower respiratory tract). The organ most affected by this virus is the lungs: SARS-CoV-2 enters host cells by binding to the enzyme angiotensin-converting enzyme 2 (ACE2), which is abundant in type II alveolar cells located in the lungs. The virus binds to and enters human cells by using its surface spike glycoprotein (spike protein) to bind ACE2 on the host cell surface. As the disease develops in the lungs, respiratory failure can occur and death may result. SARS-CoV-2 may also be responsible for respiratory failure by invading the brain stem since similar coronaviruses have been found to enter the central nervous system (CNS). Although SARS-CoV-2 has been detected in cerebrospinal fluid of the deceased, the exact mechanism of CNS invasion remains unknown. The virus can also enter cells in the gastrointestinal organs because ACE2 is expressed in the glandular cells of gastric, duodenal and rectal epithelium as well as endothelial cells of the small intestine. Finally, SARS-CoV-2 can cause myocardial injury and damage to the cardiovascular system.

SARS-CoV-2 is closely related to the original virus known as SARS-CoV. The protein makeup of SARS-CoV-2 includes membrane glycoprotein (M), envelope protein (E), spike protein (S), and the nucleocapsid protein (N). The spike protein is a glycoprotein that is split into two parts (S1 and S2). The spike protein S1 subunit catalyzes attachment to the mammalian cell surface while the S2 subunit promotes fusion with the target membrane.

Cell Biolabs' SARS-CoV-2 Spike Protein S1 ELISA Kit is an enzyme immunoassay developed for the detection and quantitation of SARS-CoV-2 Spike Protein (S1 subunit). The kit has a detection sensitivity limit of 31.3 ng/mL spike protein. Each kit provides sufficient reagents to perform up to 96 assays including standard curve and unknown samples.

Related Products

- 1. VPK-5145: SARS-CoV-2 Nucleocapsid ELISA Kit
- 2. VPK-5146: QuickTiter AAV Titer ELISA Kit
- 3. VPK-5154: West Nile Virus Envelope Protein ELISA Kit
- 4. VPK-107: QuickTiter™ Lentivirus Titer Kit (Lentivirus-associated p24 ELISA)
- 5. VPK-109: QuickTiterTM Adenovirus Titer Immunoassay Kit
- 6. VPK-112: QuickTiterTM Lentivirus Quantitation Kit
- 7. VPK-120: QuickTiterTM Retrovirus Quantitation Kit
- 8. VPK-140: ViraBindTM AAV Purification Kit
- 9. VPK-145: QuickTiterTM AAV Quantitation Kit
- 10. VPK-5112: PureVirus™ Adenovirus Purification Kit



Kit Components

Box 1 (shipped at room temperature)

- 1. <u>Anti-SARS-Cov-2 Spike S1 Ab Coated Plate</u> (Part No. 51551B): One 96-well strip plate (8 x 12).
- 2. Biotinylated Anti-Spike Protein S1 Ab (1000X) (Part No. 51552C): One 10 μL vial.
- 3. Streptavidin-Enzyme Conjugate (Part No. 310803): One 20 µL vial.
- 4. Assay Diluent (Part No. 310804): One 50 mL bottle.
- 5. <u>Triton X-100 Solution</u> (Part No. 310805): One 15 mL bottle containing 5% Triton X-100 in TBS.
- 6. 10X Wash Buffer (Part No. 310806): One 100 mL bottle.
- 7. Substrate Solution (Part No. 310807): One 12 mL amber bottle.
- 8. Stop Solution (Part. No. 310808): One 12 mL bottle.

Box 2 (shipped on blue ice packs)

1. <u>SARS-CoV-2 Spike S1 Standard</u> (Part No. 51553D): One 25 μL vial of 200 μg/mL recombinant SARS-CoV-2 Spike S1 Protein.

Materials Not Supplied

- 1. SARS-CoV-2 Samples
- 2. 10 µL to 1000 µL adjustable single channel micropipettes with disposable tips
- 3. 50 µL to 300 µL adjustable multichannel micropipette with disposable tips
- 4. Multichannel micropipette reservoir
- 5. Microplate reader capable of reading at a wavelength of 450 nm

Storage

Upon receipt, store the SARS-CoV-2 Spike S1 Standard at -80°C and avoid multiple freeze/thaw cycles. Store the Biotinylated Anti-SARS-CoV-2 Spike S1 Antibody at -20°C or -80°C. Store all other components at 4°C.

Preparation of Reagents

- 1X Wash Buffer: Dilute the 10X Wash Buffer to 1X with deionized water. Stir to homogeneity.
- Biotinylated Anti-SARS-CoV-2 Antibody and Streptavidin-Enzyme Conjugate: Immediately before use dilute the Biotinylated Anti-SARS-CoV-2 Antibody and the Streptavidin-Enzyme Conjugate 1:1000 with Assay Diluent. Do not store diluted solutions.



Preparation of Standard Curve

1. Prepare a dilution series of Spike S1 standards in the concentration range of 0 to 2000 ng/mL into Assay Diluent (Table 1).

Standard	200 μg/mL Spike S1		
Tubes	Standard (µL)	Assay Diluent (µL)	Spike S1 (ng/mL)
1	5	495	2000
2	250 of Tube #1	250	1000
3	250 of Tube #2	250	500
4	250 of Tube #3	250	250
5	250 of Tube #4	250	125
6	250 of Tube #5	250	62.5
7	250 of Tube #6	250	31.3
8	0	250	0

Table 1. Preparation of Spike S1 Standards

2. Transfer 225μL of each dilution to a microcentrifuge tube containing 25 μL of Triton X-100 Solution. Perform the assay as described in Assay Instructions.

Preparation and Inactivation of Samples

- 1. (Optional) Dilute viral supernatant in culture medium as needed. For unknown samples we recommend several dilutions for each sample. Include culture medium as a negative control.
- 2. Transfer 225 μ L of each sample to a microcentrifuge tube containing 25 μ L of Triton X-100 Solution. Vortex well.
- 3. Incubate 30 minutes at room temperature.

Assay Protocol

- Add 100 μL of Spike S1 unknown sample, standard, or blank to the Anti-SARS-CoV-2 Spike S1
 Antibody Coated Plate. Each Spike S1 unknown sample, standard and blank should be assayed in duplicate.
- 2. Incubate at room temperature for 1 hour on an orbital shaker.
- 3. Wash microwell strips 3 times with 250 μ L 1X Wash Buffer per well with thorough aspiration between each wash. After the last wash, empty wells and tap microwell strips on absorbent pad or paper towel to remove excess 1X Wash Buffer.
- 4. Add 100 μL of the diluted Biotinylated Anti-Spike S1 antibody to each well. Incubate at room temperature for 1 hour on an orbital shaker.



- 5. Wash the strip wells 3 times according to step 3 above.
- 6. Add 100 μL of the diluted Streptavidin-Enzyme Conjugate to each well. Incubate at room temperature for 1 hour on an orbital shaker.
- 7. Wash the strip wells 3 times according to step 3 above. Proceed immediately to the next step.
- 8. Warm Substrate Solution to room temperature. Add 100 μL of Substrate Solution to each well, including the blank wells. Incubate at room temperature on an orbital shaker. Actual incubation time may vary from 2-30 minutes.
 - Note: Watch plate carefully; if color changes rapidly, the reaction may need to be stopped sooner to prevent saturation.
- 9. Stop the enzyme reaction by adding 100 μL of Stop Solution into each well, including the blank wells. Results should be read immediately (color will fade over time).
- 10. Read absorbance of each microwell on a spectrophotometer using 450 nm as the primary wave length.

Example of Results

The following figures demonstrate typical results with the SARS-CoV-2 Spike S1 ELISA Kit. One should use the data below for reference only. This data should not be used to interpret actual results.



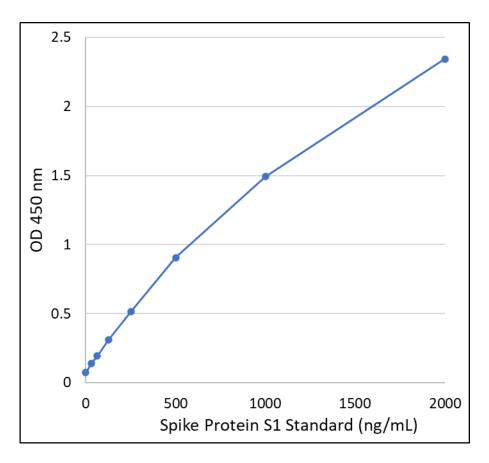


Figure 1: SARS-CoV-2 Spike Protein S1 ELISA Kit Standard Curve.

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