Product Manual

SARS-CoV-2 Nucleocapsid ELISA Kit

Catalog Number

VPK-5145	96 assays
VPK-5145-5	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 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 N Protein is an RNA binding protein which binds the RNA genome of the virus and packages it into a long helix-containing nucleocapsid structure called a ribonucleoprotein (RNP) complex. The N protein has been shown to be very important for keeping the RNA in a highly ordered conformation in order to facilitate replication and transcription of the viral genome. In addition, the N protein is responsible for regulating interactions with the host such as actin reorganization, apoptosis, and cell cycle regulation in host cells.

Cell Biolabs' SARS-CoV-2 Nucleocapsid ELISA Kit is an enzyme immunoassay developed for the detection and quantitation of SARS-CoV-2 N Protein. The kit has a detection sensitivity limit of 1.25 ng/mL N protein. Each kit provides sufficient reagents to perform up to 96 assays including standard curve and unknown samples.

Related Products

1. VPK-107: QuickTiter™ Lentivirus Titer Kit (Lentivirus-associated p24 ELISA)
2. VPK-109: QuickTiter™ Adenovirus Titer Immunoassay Kit
3. VPK-112: QuickTiter™ Lentivirus Quantitation Kit
4. VPK-120: QuickTiter™ Retrovirus Quantitation Kit
5. VPK-140: ViraBind™ AAV Purification Kit
6. VPK-145: QuickTiter™ AAV Quantitation Kit
7. VPK-5112: PureVirus™ Adenovirus Purification Kit

Kit Components

Box 1 (shipped at room temperature)

1. Anti-SARS-Cov-2 Nucleocapsid Ab Coated Plate (Part No. 51451B): One 96-well strip plate (8 x 12).
2. Biotinylated Anti-SARS-CoV-2 Nucleocapsid Ab (1000X) (Part No. 51452C): 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. Triton X-100 Solution (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. SARS-CoV-2 Nucleocapsid Standard (Part No. 51453D): One 50 µL vial of 8 µg/mL recombinant SARS-CoV-2 Nucleocapsid Protein in TBST plus BSA.

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 Nucleocapsid Standard at -80ºC and avoid multiple freeze/thaw cycles. Store the Biotinylated Anti-SARS-CoV-2 Antibody at -20ºC. Store all other components at 4ºC.

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 Nucleocapsid standards in the concentration range of 0 to 80 ng/mL into Assay Diluent (Table 1).

Standard Tubes	8 µg/mL Nucleocapsid Standard (µL)	Assay Diluent (µL)	Nucleocapsid (ng/mL)
1	5	495	80
2	250 of Tube #1	250	40
3	250 of Tube #2	250	20
4	250 of Tube #3	250	10
5	250 of Tube #4	250	5
6	250 of Tube #5	250	2.5
7	250 of Tube #6	250	1.25
8	0	250	0

Table 1. Preparation of Nucleocapsid 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 µL of each sample to a microcentrifuge tube containing 25 µL of Triton X-100 Solution. Vortex well.
3. Incubate 30 minutes at room temperature.

Assay Protocol

1. Add 100 µL of nucleocapsid unknown sample, standard, or blank to the Anti-SARS-CoV-2 Nucleocapsid Antibody Coated Plate. Each nucleocapsid 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 µL 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-SARS-CoV-2 nucleocapsid 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 Nucleocapsid ELISA Kit. One should use the data below for reference only. This data should not be used to interpret actual results.

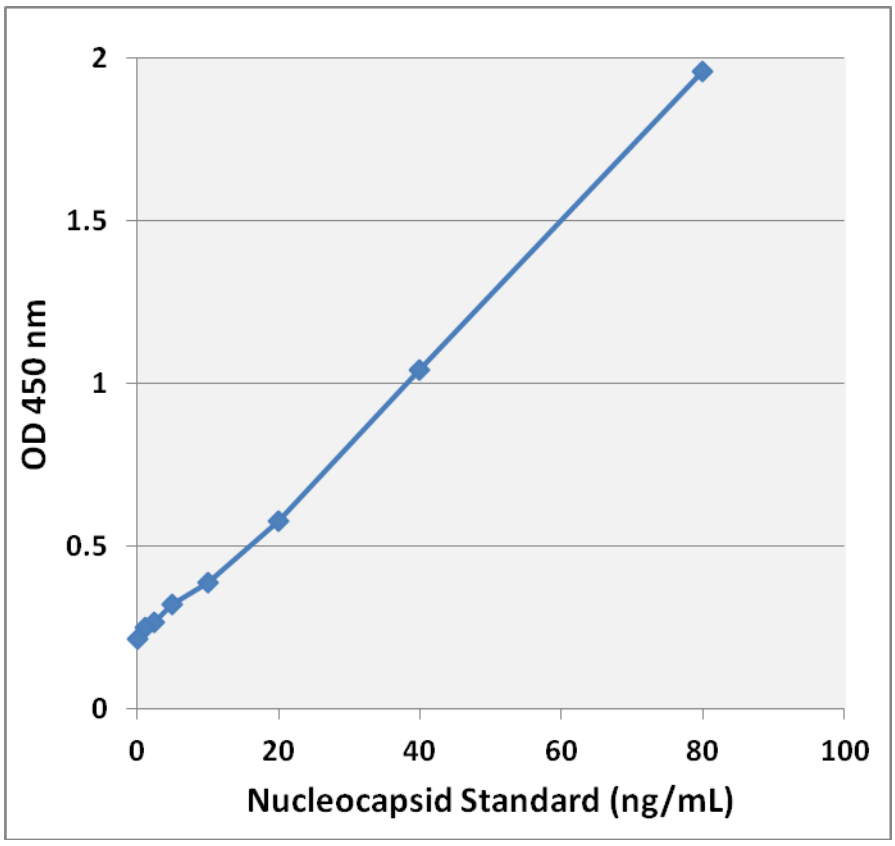

Figure 1: SARS-CoV-2 Nucleocapsid ELISA Kit Standard Curve.

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Contact Information

Cell Biolabs, Inc.
7758 Arjons Drive
San Diego, CA 92126
Worldwide: +1 858-271-6500
USA Toll-Free: 1-888-CBL-0505
E-mail: tech@cellbiolabs.com
www.cellbiolabs.com