MANUAL

COVID-19 Nucleocapsid ELISA Kit

Catalog Number CV5001

For Research Use ONLY
LifeSensors, Inc., 271 Great Valley Parkway, Malvern PA 19355 • (p) 610.644.8845 (f) 610.644.8616
techsupport@lifesensors.com • www.lifesensors.com • sales@lifesensors.com

BACKGROUND

Coronaviruses represent a family of viruses grouped together based on their crown-like appearance by electron microscopy. This classification has since been confirmed by unique features of the chemistry and replication of these viruses. Coronaviruses are the pathogenic agents for different illnesses such as the common cold, severe acute respiratory syndrome (SARS) and Middle East respiratory syndrome (MERS). In 2019, a new coronavirus, coronavirus 2 (SARS-CoV-2) was identified as the cause of a disease outbreak that originated in China causing severe acute respiratory syndrome. In March 2020, the World Health Organization (WHO) declared the COVID-19 outbreak a pandemic. Coronavirus disease (CoVID-19) is highly infectious disease primarily transmitted through direct contact with an infected person via droplets from sneezing or coughing.

Structurally, coronaviruses are spherically enveloped particles containing single-stranded (positive-sense) RNA associated with a nucleoprotein (N protein). The resulting helical nucleocapsid is anchored to the membrane protein. The viral envelope bears club-shaped glycoprotein projections in the form of S glycoproteins, which play a crucial role in the infection process of the host cell. Following the attachment of the viral S protein to host receptors, the virus enters the host cell via endocytosis. Once the Fusion of virus membrane with the endosomal membrane occurs, viral ssRNA(+) is released into the cytoplasm. The viral RNA hijacks the protein expression machinery of the infected cell for the purposes of replicating as well as producing viral proteins that are needed for new virion assembly, which exit the infected cell through exocytosis. Structural proteins such as the N protein are encoded by sub-genomic mRNAs. The N protein is required for coronavirus RNA synthesis and has RNA chaperone activity that may be involved in template switch. Nucleocapsid protein is not only the most abundant of the coronavirus proteins but is also a highly immunogenic phosphoprotein. More importantly, because its amino acid sequence is highly conserved, the coronavirus N protein represents an ideal diagnostic tool and thus, the basis of LifeSensors' SARS-CoV-1 & CoV-2 ELISA Assay.

SAFETY AND PRECAUTIONS

Personal protective equipment (PPE) within a biological safety cabinet is needed and all laboratory safety guidelines need to be followed when handling biological samples that may or may not contain the virus.

Handle laboratory waste from testing suspected or confirmed COVID-19 patient specimens as all other biohazardous waste in the laboratory. Currently, there is no evidence to suggest that this laboratory waste needs any additional packaging or disinfection procedures.

ABOUT THE ASSAY

In order to control any pandemic, the scientific community not only needs to develop vaccines and viral inhibitors, but it also needs to be equipped with an accurate viral antigen detection assay. Currently, viral detection during the SARS-CoV-2 pandemic utilizes real time reverse transcriptase polymerase chain reaction (rRT-PCR) assay that amplifies and detects the viral RNA. The rRT-PCR assay requires several days to generate test results. In addition to that, an isothermal amplification method has been approved by the FDA for the purpose of faster testing. Although these tests are helpful in differentiating between infected and non-infected individuals, they do present some challenges. Both types of assay have low specificity and low sensitivity. They are also not compatible with screening of a large population that leads to the neglect of the asymptomatic infected population. For all these reasons, LifeSensors focused on developing an antibody- and viral protein-based serological screening tool for a fast and accurate detection of SARS-CoV-2 in the form of the SARS CoV-1 & CoV-2 detection kit. It is a high-throughput assay that employs a standard sandwich ELISA format for the detection of the highly conserved N antigen. The kit may be used for quantifying natural and recombinant SARS CoV and Cov-2 nucleocapsids.

ASSAY PROCEDURE SUMMARY

Read the manual thoroughly before using the product

Collect samples.

Prepare all reagents, standards and samples as written.

Add 100 µl standard or sample to each well.
Incubate 2.5 hours at room temperature.

Wash 4 times with PBS-T.

Add 100 µl of Detection Antibody Solution to each well.

Wash 4 times with PBS-T.

Add 200 µl of Substrate Solution to each well.
Incubate 20 minutes at room temperature.

Add 50 µl of Stop Solution to each well.

Read plate at 450 nm within 30 minutes

BENEFITS

1. Absolute quantification values of natural and recombinant nucleocapsid proteins.
2. Compatible with detection of nucleocapsid proteins in patient serum/plasma samples.
3. Plate-based format is amenable to high-throughput screening (HTS). 24 samples in triplicate can be tested per plate.
4. Detection of the N protein at 1.5 pM.
5. The kit is compatible with colorimetric or chemiluminescence-based detections.
6. Highly Reproducible (CV < 10)

SUGGESTED USES

1. Detection of N protein for both SARS-CoV and SARS-CoV-2.
2. Quantification of natural nucleocapsid proteins (Patient Serum/Plasma Samples).
3. Quantification of recombinant nucleocapsid proteins (Cell Lysate).

COMPONENTS

Store all materials at 2 to 8°C, avoid repeated freeze/thaw cycles. All components (except for the microtiter plate) are stable for 6 months.

1. **1 x 96-well Microtiter Strip Plate**

   Plates are pre-coated with a Rabbit polyclonal nucleocapsid antibody. **(Pre-coated plate must be used within 1 week of receiving the kit when stored at 4°C. Pre-coated plate is stable for up to 1 year when stored at - 20°C).** Use strips as needed.

2. **SARS-CoV-2 Nucleocapsid Protein Standard**

   Size: 30,000 pg

   State: Lyophilized

3. **Detection Antibody**

   HRP-labelled Rabbit Monoclonal nucleocapsid antibody

   Size: 100 µL (200X)

4. **Standard Dilution Buffer**

   Size: 1 x 5 mL

5. **Antibody Dilution Buffer Concentrate**

   Size: 1 x 12 mL (5X)

6. **Color Reagent A**

   Size: 1 x 12 mL

7. **Color Reagent B**

   Size: 1 x 12 mL

8. **Stop Solution**

   Size: 1 x 8 mL (2N Sulfuric Acid)

9. **4 x Plate Sealers**

10. **Strip Plate Holder**

ADDITIONAL ITEMS REQUIRED

1. Phosphate Buffer Saline (PBS)
2. PBS with 0.1% Tween 20 (PBS-T)
3. Optical Density Microplate Reader

   It must be capable of measuring absorbance at 450 nm.
4. 1.5 ml snap cap tubes
5. 15 ml centrifuge tubes

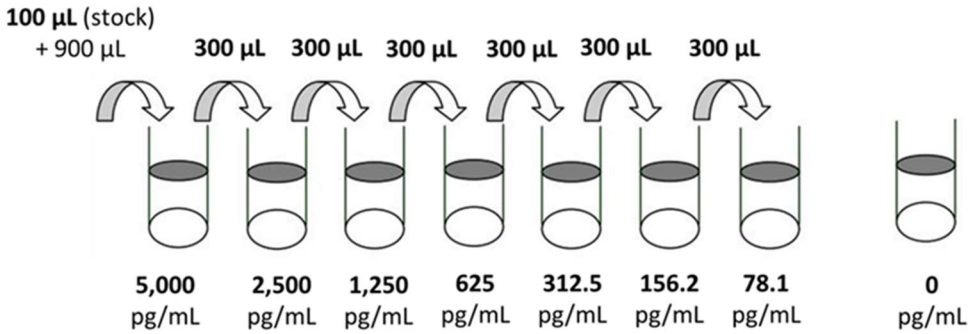
SOLUTIONS PREPARATION

Make a plate map and calculate the volume needed for each solution

Standard Curve Solutions

1. The nucleocapsid standard stock solution (50,000 pg/mL) is prepared by adding 600 µL of dilution buffer to the lyophilized nucleocapsid standard. Vortex.
2. Add 100 µL of the nucleocapsid standard stock solution to 900 µL of dilution buffer to make a standard concentration of 5,000 pg/mL. Vortex.

   Add 300 µL of the 5,000 pg/mL nucleocapsid standard solution to 300 µL of dilution buffer to make a standard concentration of 2,500 pg/mL. Vortex. Continue performing the same serial dilution for a total of 7 points as part of the calibration curve (See Below) while the dilution buffer alone corresponds to a "zero standard" (0 pg/mL). Vortex between serial dilutions.



Antibody Dilution Buffer

1. Add 1 mL of Antibody Dilution Buffer Concentrate to 4 mL of PBS. Vortex.

Detection Antibody Solution

1. Perform a 200-fold dilution of the Detection Antibody in the Antibody Dilution Buffer (e.g. add 50 µL of Detection Antibody in 10 mL of Antibody Dilution Buffer). Mix Gently.

Substrate Solution

1. Mix equal parts of Color Reagent A and Color Reagent B.
   NOTE: Prepare the Substrate Solution right before use. Keep away from light by covering the tube with aluminum foil. Do not store this solution for longer than 30 min.

PROTOCOLS

Sample Collection and Storage

As the coronavirus inactivation data is still evolving, use caution and the latest published methods of inactivation.

The COVID-19 Nucleocapsid ELISA Kit is compatible with the detection of nucleocapsid in other bodily fluids (e.g., saliva, urine…). However, the kit has only been validated for human serum.

1. **Cell Lysate** – Lyse the cells in 0.5% IGEPAL CA-630. Isolate the soluble fraction by centrifuging at 10,000 x g for 20 min at 4°C. Assay the clarified lysate immediately or aliquot and store samples at -20°C or at lower temperature. Avoid repeated freeze-thaw cycles.
2. **Serum** - Use a serum separator tube. Allow the sample to clot for 30 minutes before centrifuging for 15 minutes at 1000 x g. Assay the separated serum immediately or aliquot and store samples at -20°C or at lower temperature. Avoid repeated freeze-thaw cycles.
3. **Saliva** - Collect saliva in a tube and centrifuge for at 10,000 x g for 5 min at 4°C. Assay the aqueous layer immediately or aliquot and store samples at -20°C or at lower temperature. Avoid repeated freeze-thaw cycles.
4. **Urine** – Collect urine and centrifuge at 10,000 x g for 2 min at 4°C.

NOTE: For the inactivation of the virus in all samples use NP-40 at a final concentration of 1% or a commercial viral buffer (e.g., Buffer AVL from Qiagen). Vortex well before proceeding to the sample dilution step.

Sample Dilution

1. For the purpose of measuring nucleocapsid levels, it is recommended to test the non-diluted fraction in addition to a 1/10 and a 1/100 dilutions (in dilution buffer) as a starting point.
2. It is recommended to perform serial dilutions for each point to be measured in triplicate.

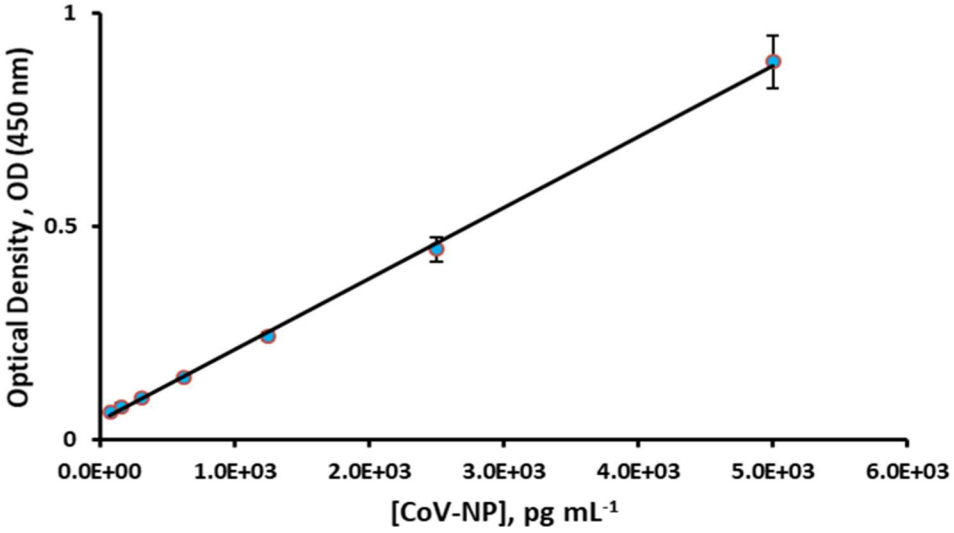
Nucleocapsid Detection (Suggested Protocol)

1. As directed in previous sections, prepare all reagents, standards, and samples.
2. Determine the number of coated microplate wells required (based upon the number of reactions to be run) and transfer strips to the extra plate holder. Allow coated plate wells to equilibrate to room temperature.
   NOTE: Place any unused strips back in the foil bag and store at 2-8°C or at -20°C.
3. Start by washing the plate 1 time with PBS-T prior to adding standard reagents.
   NOTE: Plates can be washed by an automated plate washer or by manual washing. In the latter case, complete removal of all the wash buffer by blotting against adsorbent paper towel is essential for reproducible results.
4. Add 100 µL of nucleocapsid standards, controls/blanks and of diluted samples to the designated wells according to your plate map. Allow the plate to incubate for 2 h at room temperature (20-25°C).
   NOTE: Use the provided plate sealers to cover the plate between each incubation in order to avoid the evaporation of reagents and to protect the samples from light.
5. Aspirate the protein solutions. Wash the unbound fraction of the samples/reagents off the plate with 200 µL of PBS-T per well. Repeat this step 3 more times.
   NOTE: Ensure no residual wash buffer is left after final aspiration by blotting against a fresh paper towel.
6. Add 100 µL of the Detection Antibody Solution to each well and incubate for 1 h at room temperature and cover the plate with plate sealer.
7. Repeat Step 5.
8. Add 200 µL of Substrate Solution to each well and incubate for 20 min at room temperature using a plate sealer. Avoid exposing to light.
9. Add 50 µL of Stop Solution and gently tap the plate to mix the color uniformly.
10. Measure the optical density at 450 nm on a plate reader within 30 mins of stopping the reaction.

11. Plot the standard calibration curve by graphing the optical density value obtained for each standard to Its known concentration.
12. Fit a line that best represents the data. The line should have the general equation of OD = m (Protein Concentration) + b
13. To determine the protein concentration of the tested samples, extrapolate the protein concentration value using the obtained optical density using the above equation.
14. Multiply the calculated protein concentration by the dilution factor used to dilute the sample.

EXAMPLE OF A NUCLEOCAPSID STANDARD CURVE

SARS-CoV Nucleocapsid protein solutions at different concentrations were prepared by serial dilution. LifeSensors' COVID-19 Nucleocapsid ELISA Kit was used to detect and measure the resulting signal and more importantly to verify the linearity of the Standard Calibration Curve (See Below).



SPIKE AND RECOVERY TEST

SARS-CoV Nucleocapsid protein standards at 3 different concentrations were spiked in human plasma. LifeSensors' COVID-19 Nucleocapsid ELISA Kit was used to detect and measure the nucleocapsid protein concentration. The percentage of recovery was calculated (See Table Below). Standard FDA guideline consider the recovery of an assay validated when the percentage of recovery lies between 80 and 120%.

Spike Concentration (pg/mL)	Recovered Concentration (pg/mL)	Percentage of Recovery (%)
1000.0	1113.0	111.3
500.0	402.0	80.4
250.0	262.0	104.8

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6. US Food & Drug Administration. Emergency Use Authorizations. (2020).
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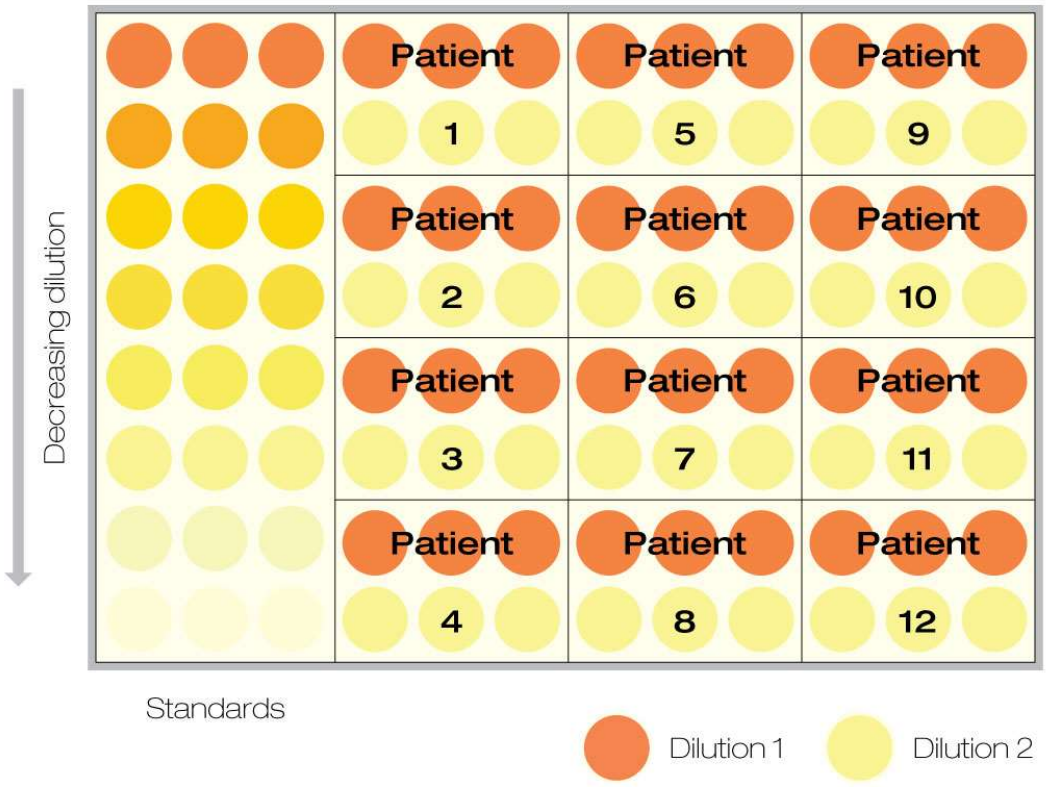
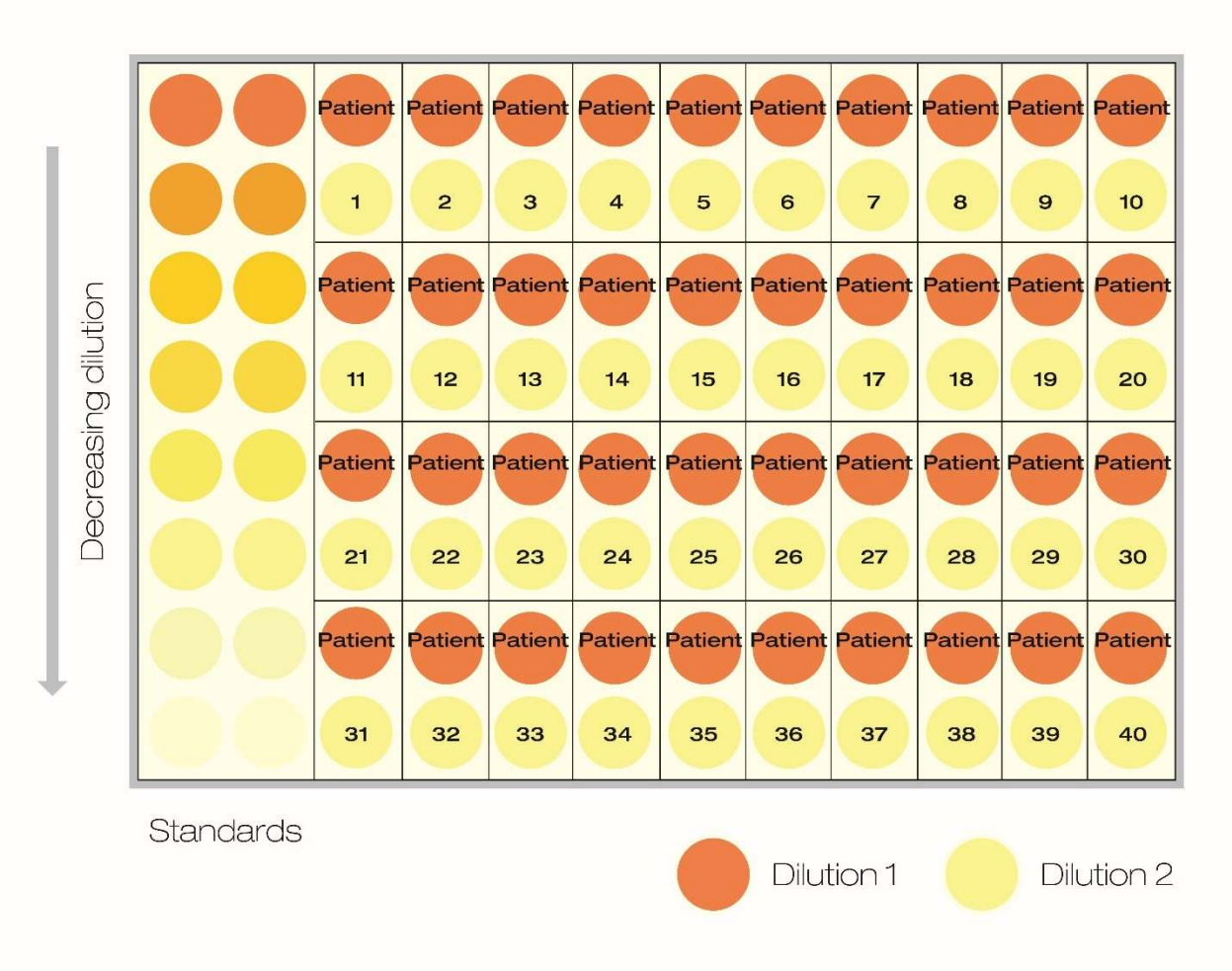
CONTROLS TO MINIMIZE ASSAY VARIANCE

1. Avoid subjecting the plate to repeated freeze-thaw cycles if you store it at - 20°C.
2. Create a detailed plate map to avoid confusion and ensure trackability of samples.
3. Run the appropriate blanks (no protein samples).
4. Run a standard calibration curve for every tray of samples assayed.
5. Store the Color Reagents away from light.



COVID-19 Nucleocapsid ELISA Plate Map. Nucleocapsid standards and patient samples are tested in a triplicate format. Different concentration of nucleocapsid standard proteins are generated via serial dilutions and are deposited on the plate (left side) vertically from the highest concentration (bright red wells) to the blank (faded yellow wells). A single COVID-19 Nucleocapsid ELISA plate allows for testing two different dilutions of 12 different patient samples in a triplicate format. See above figure for the suggested location of the samples on the plate (right side of the standards).



COVID-19 Nucleocapsid ELISA Plate Map. Nucleocapsid standards and patient samples are tested in a triplicate format. Different concentration of nucleocapsid standard proteins are generated via serial dilutions and are deposited on the plate (left side) vertically from the highest concentration (bright red wells) to the blank (faded yellow wells). A single COVID-19 Nucleocapsid ELISA plate allows for testing two dilutions of 40 different patient samples in a single sample format. See above figure for the suggested location of the samples on the plate (right side of the standards).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