

SARS-CoV-2 Nucleocapsid ELISA Kit

(Catalog # E4878-100, 96 assays, Store at 4°C)

I. Introduction:

Coronaviruses are a diverse group of RNA viruses that infect humans, mammals, and avian species. Previously there were seven known human coronaviruses, of which two were considered highly pathogenic and caused fatal respiratory illness, namely, SARS-CoV and MERS-CoV. A novel SARS-CoV-2 virus emerged at the end of 2019 that resulted in COVID-19 disease, which proved to be deadlier than its predecessors and resulted in a worldwide pandemic. SARS-CoV-2 is a large single-stranded, positive-sense RNA that is genetically similar to SARS-CoV (80%). It consists of 4 structural proteins required for viral assembly: Spike (S), Envelope (E), Membrane (M), and Nucleocapsid (N) protein. The Nucleocapsid is the most abundant protein in SARS-CoV-2. Due to its critical role in viral assembly, the Nucleocapsid protein could serve as a potential target for vaccine and drug treatments. BioVision's SARS-CoV-2 Nucleocapsid ELISA Kit is designed to quantitatively measure the amount of Nucleocapsid proteins in bronchoalveolar lavage fluid and nasopharyngeal swab samples. The assay is based on the Sandwich ELISA principle. Test samples, Standards, and Biotin-conjugated antibody are added to the wells pre-coated with Nucleocapsid antibody and then washed with Wash Buffer. The HRP-Streptavidin is added and any unattached conjugates are washed off using Wash Buffer. The HRP enzymatic reaction is detected by the addition of TMB-substrate. Finally, the reaction is terminated with an acidic stop solution. The color developed is proportional to the concentration of Nucleocapsid in the sample or standard.

II. Features and Benefits:

- Detection range: 12.5 – 80 ng/ml
- Sensitivity: < 1 ng/ml
- Assay Precision: Intra-Assay CV < 12% and Inter-Assay CV < 15%
- This Sandwich ELISA is highly sensitive and highly specific for the detection of Nucleocapsid protein in bronchoalveolar lavage fluid and nasopharyngeal samples

III. Sample Type:

Bronchoalveolar lavage fluid and nasopharyngeal samples

IV. Kit Contents:

Components	E4878-100	Part Number
Capture ELISA plate	8 x 12 Strips	E4878-100-1
SARS-CoV-2 NP Standard (160 ng)	1 bottle	E4878-100-2
Standard/Sample Dilution Buffer	25 ml	E4878-100-3
NP Biotin Conjugated Antibody	120 µl	E4878-100-4
Reagent Dilution Buffer	25 ml	E4878-100-5
HRP-Streptavidin Conjugate (SABC)	120 µl	E4878-100-6
TMB Substrate Solution	12 ml	E4878-100-7
Stop Solution	6 ml	E4878-100-8
Wash Buffer (20X)	25 ml	E4878-100-9
Plate Sealer	4	E4878-100-10

V. User Supplied Reagents and Equipment:

- Microplate reader capable of measuring absorbance at 450 nm
- 37°C incubator
- Precision pipettes with disposable pipette tips
- Distilled or deionized water
- Clean eppendorf tubes for preparing standards or sample dilutions
- Absorbent paper

VI. Storage and Handling:

Store the unopened kit at 4°C for 6 months. If the kit is opened, it must be used within 1 month. Do not use the kit beyond the expiration date

VII. Reagent and Sample Preparation:

Note: Prepare fresh reagents before the start of the experiment

Before using the kit, spin tubes and bring down all components to the bottom of tubes
 Bring all reagents to room temperature prior to use

1. **Biotin-labeled Antibody working solution:** Prepare a working stock solution of SARS-CoV-2 NP Biotin-conjugate antibody by diluting the antibody in Reagent Dilution Buffer at 1:100. For example, dilute 100 µl of Biotin-conjugate antibody in 9,900 µl Reagent Dilution Buffer to prepare a 10 ml working stock of Biotin conjugated antibody solution.
2. **HRP-Streptavidin Conjugate (SABC):** Prepare a working stock solution of HRP-Streptavidin conjugate by diluting the conjugate in Reagent Dilution Buffer at 1:100. For example, dilute 100 µl of HRP-Streptavidin conjugate in 9,900 µl Reagent Dilution Buffer to prepare a 10 ml working stock of HRP-Streptavidin conjugate solution.
3. **Wash Buffer:** Dilute 20X Wash Buffer to 1X by adding 20 ml of 20X Wash Buffer to 380 ml deionized/distilled water to prepare 400 ml of 1X Wash Buffer solution. If crystals present in the 20X Wash Buffer, warm it at room temperature. Mix it gently to dissolve the crystals.
4. **Standard Preparation:**
 - Add 2 ml Standard/Sample Dilution Buffer to reconstitute the SARS-CoV-2 NP standard. The concentration of the standard

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after reconstitution will be 80 ng/ml. This can be labeled as High Standard. Prior to making dilutions, mix the standard solution well and allow it to sit for 10 minutes.

- Label 7 tubes as 40 ng/ml, 20 ng/ml, 10 ng/ml, 5 ng/ml, 2.5 ng/ml, 1.25 ng/ml and 0 ng/ml respectively. Add 400 µl of the Standard/Sample Diluent Buffer into Tubes 1-7. Pipette out 400 µl from 80 ng/ml Stock Solution and add to Tube 1. Mix thoroughly.
- Transfer 400 µl from Tube 1 to Tube 2 and mix well. Transfer 400 µl from Tube 2 to Tube 3 and mix and so on. Tube 7 will only have 400 µl of the Standard/Sample Dilution Buffer which will serve as Zero Standard (0 ng/ml).

VIII. Assay Protocol:

Note: Bring all reagents and samples to room temperature 30 minutes prior to the assay.

It is recommended that all standards and samples be run at least in duplicate.

A standard curve should be run for each assay.

1. Prepare all reagents, samples and standards as instructed in **section VII**.
2. Any unused microplate strips must be removed from the plate frame and returned to the foil pouch that contains desiccant pack and must be resealed.
3. Add 100 µl of each **Standards** (0 – 80 ng/ml) or **Samples** into appropriate wells. Cover the wells with the Plate sealer and incubate for 1 hour at 37°C.
4. Remove the Plate sealer and aspirate the plate contents. Wash the plate 3 times with 300 µl **1X Wash Buffer**. Wash by filling each well with **1X Wash Buffer** using a multi-channel pipette or auto-washer and then remove all residual **Wash Buffer** from the wells by aspiration. After the last wash, remove any remaining **Wash Buffer** by aspirating or decanting. Tap the plate on absorbent filter papers.
5. Add 100 µl of **NP Biotin-conjugated antibody work solution** into the above wells. Seal the plate and incubate at 37°C for 30 minutes.
6. Discard the solution and wash 3 times with **1X Wash Buffer**.
7. Add 100 µl of **SABC working solution** into each well, cover the plate and incubate at 37°C for 30 minutes.
8. Discard the solution and wash 3 times with **1X Wash Buffer** as step 6.
9. Add 100 µl of **TMB substrate** into each well, cover the plate and incubate at 37 °C in dark for 10 minutes. Protect the plate from light
10. Add 50 µl of **Stop Solution** to each well. The color should change from blue to yellow. If the color changes to green or appears non-uniform, gently tap the plate to ensure thorough mixing. Read result at 450 nm within 30 minutes. If wavelength correction is available, then set to 540 or 570 nm. If wavelength correction is not available, then subtract 540 nm/ 570 nm from 450 nm. The subtraction will correct for any optical interference in the plate. If wavelength correction is not performed, the readings at 450 nm may be higher and less accurate.

IX. Calculation:

For calculation, (the relative O.D.450) = (the O.D.450 of each well) – (the O.D.450 of Zero well). The standard curve can be plotted as the relative O.D.450 of each standard solution (Y) vs. the respective concentration of the standard solution (X). The target concentration of the samples can be interpolated from the standard curve. If the samples measured were diluted, multiply the dilution factor to the concentrations from interpolation to obtain the concentration before dilution.

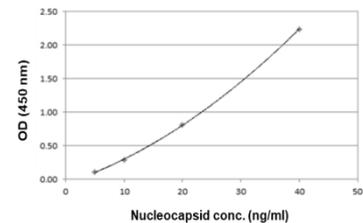


Figure: Typical Standard Curve and OD values: These standard curves are for demonstration only. A standard curve must be run with each assay.

X. Related Products:

- Coronavirus Rapid RT-qPCR Detection Kit (K1461)
- SARS-CoV-2 Nucleoprotein IgG Antibody ELISA Kit
- Coronavirus (SARS-CoV-2) PCR Detection Kit (K1460)
- SARS-CoV-2 IgG ELISA Kit
- SARS-CoV-2 IgM ELISA Kit