

SARS-CoV-2 Nucleocapsid IgG ELISA Kit

10/20

(Catalog # E4903-100, 100 assays; Store at -20 °C)

I. Introduction:

Coronaviruses are large, lipid-enveloped, single-stranded RNA viruses known to infect a wide array of hosts, including humans, bats, mice and other mammals. Amongst the seven known human coronaviruses, MERS-CoV, SARS-CoV-1 and SARS-CoV-2 are the most pathogenic, causing severe, potentially fatal respiratory and cardiovascular symptoms. The emergence of the novel SARS-CoV-2 virus, the cause of the ongoing Coronavirus Disease 2019 (COVID-19) pandemic, has proven to be the greatest public health crisis of the 21st Century. SARS-CoV-2 has a ~30 kb genome that encodes 29 proteins, including 4 structural proteins used in virion assembly, namely the Envelope (E), Membrane (M), Spike (S) and Nucleocapsid (N) proteins. The N protein is a RNA-binding protein that maintains the viral genomic RNA in a conformation required for viral RNA transcription and replication. The N protein amino-terminal contains the RNA-binding Domain, a potential structural target for novel antiviral drugs. The N protein is a highly immunogenic antigen, with specific anti-N protein antibodies detected in patient serum 5-10 days following infection. **BioVision's SARS-CoV-2 Nucleocapsid IgG ELISA Kit** is an enzyme-linked immunosorbent assay intended for identifying the immune response to SARS-CoV-2 infection. The assay uses the nucleocapsid protein as a bait to capture anti-SARS-CoV-2 antibodies in diluted plasma or serum samples. Subsequent reaction with an enzyme-labeled anti-human-IgG conjugate and a colorimetric substrate generates color, which is proportional to the amount of anti-N antibodies. The presence of anti-SARS-CoV-2 N antibodies (IgG) is determined by comparing the absorbance (OD_{450 nm}) reading of the sample to the OD_{450 nm} reading of the negative control serum. **A positive reaction suggests prior SARS-CoV-2 infection.** Positive control serum containing a recombinant human monoclonal anti-SARS-CoV-2 N antibody is included in the kit for validation. The assay is a quick, easy and accurate method of indicating recent or prior SARS-CoV-2 infection that does not require collection or handling of infectious virus. This kit is a valuable tool to study the COVID-19 epidemiology, prevalence and morbidity.

II. Applications:

- Determination of prior SARS-CoV-2 infection
- Screening of SARS-CoV-2 infection rate in a given population
- Study of epidemiology, prevalence and morbidity of COVID-19

III. Sample Type:

- Human Serum or Plasma (Heparin or EDTA)

IV. Kit Contents:

Components	E4903-100	Cap Code	Part Number
Nucleocapsid Protein-Coated ELISA Plate	8 x 12 Strips	--	E4903-100-1
Wash Buffer (10X)	50 ml	WM	E4903-100-2
Sample Diluent	50 ml	NM	E4903-100-3
Anti-IgG-HRP Conjugate (1000X)	50 µl	Blue	E4903-100-4
Positive Control Serum	30 µl	Violet	E4903-100-5
Negative Control Serum	60 µl	White	E4903-100-6
TMB Substrate	10 ml	Amber	E4903-100-7
Stop Solution	10 ml	NM	E4903-100-8
Plate Sealing Film	2	--	E4903-100-9

V. User Supplied Reagents and Equipment:

- Multiwell microplate spectrophotometer (capable of reading absorbance at 450 nm)
- Precision multi-channel pipette and reagent reservoir

VI. Storage Conditions and Reagent Preparation:

Store the kit at -20 °C protected from light. Briefly centrifuge small vials at low speed prior to opening. Read the entire protocol before performing the experiment. Dilute concentrated reagent stock solutions to working concentration as needed immediately prior to use.

- **Nucleocapsid Protein-Coated ELISA Plate:** Do not open until ready to use. Bring to room temperature (RT) prior to opening. **After opening, immediately store the remaining unused strips at 4 °C in foil bag with desiccant** to protect the strip wells from moisture.
- **Wash Buffer (10X):** Warm to RT prior to use (if crystals are present, mix gently until the crystals are completely dissolved). Prepare 100 ml of 1X Wash Buffer by diluting 10 ml of Wash Buffer (10X) with 90 ml deionized water. The 1X Wash Buffer is stable at 4 °C for one month.
- **Anti-IgG-HRP Conjugate (1000X):** Divide the stock into aliquots if desired and store at -20 °C, protected from light. Avoid repeated freeze/thaw cycles.
- **Positive Control Serum and Negative Control Serum:** Divide into aliquots and store at -20 °C. Concentrated solution is stable for 4 freeze/thaw cycles.
- **TMB Substrate, Sample Diluent and Stop Solution:** Provided as a ready-to-use solution. Bring to RT prior to use. When not in use, reseal bottle immediately and store at 4 °C, protected from light.

VII. SARS-CoV-2 Nucleocapsid IgG ELISA Assay Protocol:

1. Sample Preparation: Note: Use proper personal protection equipment when handling biological samples.

- Collect serum or plasma samples by standard methods. We recommend using either "off-the-clot" serum (collected in tubes that are free of anticoagulants) or plasma collected with EDTA or lithium/sodium heparin. Samples exhibiting lipemia or excessive turbidity should be clarified using a non-binding 0.2 µm syringe filter (e.g. BioVision Cat # M4332) in order to eliminate lipid globules and other debris. Once collected, serum/plasma samples may be stored at -20 °C for up to one month prior to assay.

Note: Do not use serum/plasma that is hemolyzed or contaminated with red blood cells, as this may interfere with the assay.

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- b. In clean microfuge tubes, dilute serum/plasma test samples at a 1:300 ratio with Sample Diluent (for example, mix 2 µl of serum or plasma with 598 µl of Sample Diluent).

2. Assay Plate Layout and Reagent Preparation:

- a. Format the microplate for the number of serum/plasma test samples to be assayed by placing a sufficient number of wells in the plate frame, including two duplicate wells for the Negative Control and one well for the Positive Control.
- b. In clean microfuge tubes, dilute the Negative Control Serum at 1:300 ratio with Sample Diluent (for example, mix 2 µl of Negative Control Serum with 598 µl of Sample Diluent).
- c. Dilute the Positive Control Serum at 1:100 ratio with Sample Diluent (for example, mix 2 µl of Positive Control Serum with 198 µl of Sample Diluent).
- d. Add 100 µl of diluted (1:300) test samples, diluted Negative Control Serum (1:300) and diluted Positive Control Serum (1:100) to the designated wells. Mix wells and cover with plate sealing film. Incubate plate for 30 min at 25 °C with gentle orbital shaking.

3. Secondary Antibody (Anti-Human IgG) Reaction:

- a. Remove the plate sealing film. Aspirate the solution from each well add 300 µl of 1X Wash Buffer to each well. Aspirate the Wash Buffer from each well and pat it dry against clean absorbent paper. Repeat this wash step a total of 4 times.
Note: A microplate washer can be used in this step and other wash steps.
- b. Prepare a working solution of 1X Anti-IgG-HRP Conjugate by diluting the 1000X stock solution in 1X Wash Buffer (for example, mix 5 µl of 1000X Anti-IgG-HRP Conjugate with 4995 µl of 1X Wash Buffer). Prepare a sufficient amount of the 1X Anti-IgG-HRP Conjugate solution to add 100 µl per well.
Note: Make the 1X Anti-IgG-HRP Conjugate solution fresh. Do not store or reuse the diluted Anti-IgG-HRP Conjugate.
- c. Add 100 µl of 1X Anti-IgG-HRP Conjugate to each well. Mix wells and cover with plate sealing film. Incubate plate for 30 min at 25 °C with gentle orbital shaking.
- d. Aspirate the solution from each well and add 300 µl of 1X Wash Buffer to each well. Aspirate the Wash Buffer from each well and pat it dry against clean absorbent paper. Repeat this wash step a total of 4 times.

4. **Measurement:** Remove any residual Wash Buffer from the wells and add 100 µl TMB Substrate to all wells. Incubate plate (protected from light) for 10 min at 25 °C. Following the 10 min incubation period, add 100 µl Stop Solution into each well. Measure the absorbance at 450 nm in a microplate reader within 15 min of adding Stop Solution.

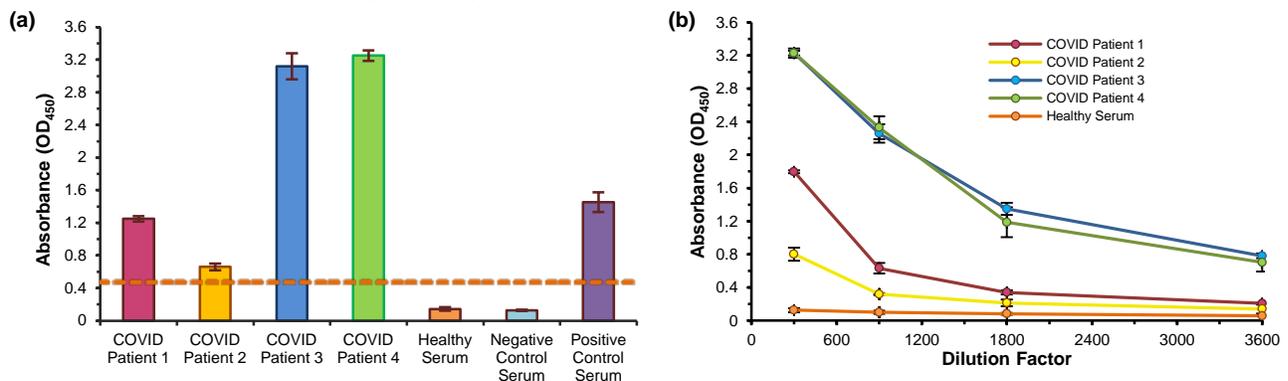
Note: The 10 min TMB reaction time must be consistent for all wells. To minimize variations in substrate incubation time, we recommend using a multichannel pipette and reagent reservoir to add TMB Substrate and Stop Solution.

5. **Calculation:** Calculate the mean absorbance (OD₄₅₀) value obtained for the Negative Control Serum (A_{NC}) and all of the Test Samples (A_{sample}). The cutoff for a presumptive positive sample (at a 1:300 dilution factor) is:

$$A_{\text{sample}} \geq 1.1 \times (A_{\text{NC}} + 0.3)$$

Note: When diluted at 1:300, the Negative Control Serum must generate an OD_{450 nm} signal less than 0.2 for the test to be valid. The Positive Control Serum should generate an OD_{450 nm} greater than 1.0 when diluted at 1:100 ratio.

If desired, positive samples may be run at further dilutions to determine the antibody titer or to calculate the area under the curve (AUC) of sample dilution curves. **Negative results do not preclude acute SARS-CoV-2 infection.** Particularly in the first week after onset of infection, patient samples may be negative for IgG. If acute infection is suspected, direct testing for SARS-CoV-2 is necessary.



Figures: (a) Detection of Anti-SARS-CoV-2 Nucleocapsid IgG antibodies in serum samples (dilution 1:300). COVID-19 patient samples were obtained from individuals that had a positive test for SARS-CoV-2 viral RNA by RT-PCR from nasopharyngeal swab specimen. All patient serum samples were collected at least 14 days following the initial onset of symptoms and 9-20 days following PCR confirmation of infection. Healthy serum was donor-pooled off-the-clot serum obtained from healthy individuals prior to the COVID-19 pandemic. The response from Negative Control Serum was used to determine the cutoff for positivity (indicated by the dashed line). (b) Serial dilutions of COVID-19 patient sera versus healthy serum. Calculated AUC values for COVID-19 patient samples were 5.96-, 3.22-, 18.65- and 17.81-fold greater than that of pre-pandemic healthy serum, respectively. Data presented are mean ± SD of triplicate or greater wells.

VII. Related Products:

SARS-CoV-2 (Spike Protein) IgG ELISA Kit (E4901)
 SARS-CoV-2 S1 Protein-ACE2 Binding Inhibitor Screening Kit (K2050)
 Coronavirus IgM/IgG Antibody Detection Card (K1463)
 Recombinant SARS-CoV-2 Nucleoprotein (P1523)

SARS-CoV-2 (Spike Protein) IgM ELISA Kit (E4902)
 Coronavirus Rapid RT-qPCR Detection Kit (K1461)
 Recombinant SARS-CoV-2 3C-like Protease (P1550)
 Human CellExp™ SARS-CoV-2 Spike Protein (P1524)

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