



SARS-CoV-2 Nucleoprotein IgG Antibody ELISA Kit

rev 02/21

(Catalog # E4821-100, 96 assays, Store at 4°C)

I. Introduction:

Severe acute respiratory syndrome (SARS) is a viral respiratory illness caused by a coronavirus called SARS-associated coronavirus (SARS-CoV). The coronavirus genome encodes a spike protein (S), an envelope protein, a membrane protein, and a nucleoprotein in this order. Nucleoprotein packages the positive strand viral genome RNA into a helical ribonucleocapsid (RNP) and plays a fundamental role during virion assembly through its interactions with the viral genome and membrane protein (M). BioVision's SARS-CoV-2 Nucleoprotein IgG Antibody ELISA Kit is based on indirect qualitative enzyme immunoassay technique. The microtiter plate provided in this kit has been pre-coated with SARS-CoV-2 Nucleoprotein. Samples are pipetted into the wells with anti-human IgG conjugated Horseradish Peroxidase (HRP). Any antibodies specific for the antigen present will bind to the pre-coated antigen. Following a wash to remove any unbound reagent, a substrate solution is added to the wells and color develops in proportion to the amount of SARS-CoV-2 Nucleoprotein IgG antibody bound in the initial step. The color development is stopped by adding stop solution and the intensity of the color is measured.

II. Application:

This ELISA kit is used for *in vitro* qualitative determination of SARS-CoV-2 Nucleoprotein IgG Antibody.

Intra-assay Precision: CV% <15%

Inter-assay Precision: CV% <15%

III. Sample Type:

Serum, Plasma

IV. Kit Contents:

Components	E4821-100	Part No.
Micro ELISA Plate	8 x 12 Strips	E4821-100-1
Negative Control	800 µl	E4821-100-2
Positive Control	800 µl	E4821-100-3
HRP Conjugate (100x)	120 µl	E4821-100-4
HRP Conjugate Diluent	20 ml	E4821-100-5
Sample Diluent	2 x 20 ml	E4821-100-6
Wash Buffer (25x)	20 ml	E4821-100-7
TMB Substrate	10 ml	E4821-100-8
Stop Solution	10 ml	E4821-100-9
Plate sealers	4	E4821-100-10

V. User Supplied Reagents and Equipment:

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

VI. Storage and Handling:

The entire kit may be stored at 4°C for up to 12 months from the date of shipment. An opened kit may be stable for 1 month at 4°C.

VII. Reagent and Sample Preparation:

Note: Bring all reagents to room temperature (20-25°C) 30 minutes before use. Before using the kit, spin tubes and bring down all components to the bottom of tubes. Finish preparing reagent 10 minutes before the assay.

1. **Wash Buffer (1X):** If crystals have formed in the concentrate, warm up to room temperature and mix gently until the crystals are completely dissolved. Dilute 20 ml of Wash Buffer (25X) into deionized water to prepare 500 ml of Wash Buffer (1X).
2. **HRP Conjugate (1x):** Centrifuge the vial before opening. HRP- conjugate requires a 100-fold dilution. A suggested 100-fold dilution is 10 µl of HRP-conjugate + 990 µl of HRP- conjugate Diluent.
3. **Sample Collection and Storage:**
 - **Serum:** Collect the sample in a serum separating tube and allow it to clot at room temperature for 2 hours or overnight at 4°C. Centrifuge at 1000 x g for 15 minutes. Collect serum and perform assay immediately. Alternatively, aliquot and store serum sample at -20°C or 80°C. Avoid freeze/thaw cycles.
 - **Plasma:** Collect the plasma sample using citrate, EDTA or heparin as an anticoagulant. Within 30 minutes of collection, centrifuge at 1000 x g for 15 minutes. Perform the assay immediately or aliquot and store the samples at -20°C. Avoid freeze/thaw cycles.
4. **Sample Preparation:**



- Dilute the serum or plasma samples with Sample Diluent (1:100) before test. The suggested 100 fold dilution can be achieved by adding 10 µl sample to 40 µl of Sample Diluent. Complete the 100 fold dilution by adding 15 µl of this solution to 285 µl of Sample Diluent.
- **Note:** Samples to be used within 5 days may be stored at 2-8°C. for long term storage, store at -20°C (≤ 1month) or -80°C (≤ 2 month) to avoid loss of bioactivity and contamination. Hemolyzed samples are not suitable for use in this assay.

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 duplicates.

1. Prepare all reagents, samples and standards as instructed in section VII. Store unused wells back to 4°C.
2. Set a Blank well without any solution.
3. Add 100 µl of **Negative Control**, **Positive Control** or **diluted Sample** per well. Cover with the plate sealer and incubate for 30 minutes at 37°C.
4. Aspirate each well and wash, repeating the process 3 times. Wash by filling each well with 200 µl of **Wash Buffer (1X)** using a squirt bottle, multi-channel pipette, manifold dispenser, or autowasher, and let it stand for 2 minutes, complete removal of liquid at each step is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against clean paper towels.
5. Add 100 µl of **HRP Conjugate (1X)** to each well (do not add in Blank!). Cover the microtiter plate with the plate sealer. Incubate for 30 minutes at 37°C.
6. Repeat the wash step for five times as described in step 4.
7. Add 90µl of **TMB Substrate** to each well. Incubate for 20 minutes at 37°C. Protect from light.
8. Add 50µl of **Stop Solution** to each well, gently tap the plate to ensure thorough mixing.
9. Read blank well as zero, determine the optical density of each well within 10 minutes, using a microplate reader set to 450 nm.

IX. CALCULATION:

For calculation of SARS-CoV-2 Nucleoprotein IgG antibody, compare the sample well with control.

- If "**Sample OD < 2.1x OD Negative control: sample is NEGATIVE**"
- If "**sample OD ≥ 2.1x OD Negative control: sample is POSITIVE**"

Typical data:

Test parameter	Specification	Test result
Positive control	≥0.6	0.867
Negative control	≤0.25	0.184
Positive rate	20 Positive	100%
Negative rate	20 Negative	100%

X. RELATED PRODUCTS:

- ACE2 (Human) ELISA Kit (E4528)
- Coronavirus (SARS-CoV-2) PCR Detection Kit (K1460)
- Coronavirus IgM/IgG Antibody Detection Card (K1463)
- Coronavirus Rapid RT-qPCR Detection Kit (K1461)