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ab276183 COVID-19 N-Protein Human IgA ELISA Kit

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COVID-19 N-Protein Human IgA ELISA Kit datasheet:
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For the semi-quantitative measurement of COVID-19 N-Protein human IgA in serum.

This product is for research use only and is not intended for diagnostic use.

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1. Overview

COVID-19 N-Protein Human IgA Human IgA ELISA Kit (ab276183) is an *in vitro* indirect ELISA for the semi-quantitative measurement of human IgA antibody against SARS-CoV-2 N protein in human serum.

Standard 96-well plates (12 strips with 8 wells/strip) are coated with the SARSCoV-2 N protein, which combines with the corresponding antibody present in a sample. When a secondary anti-human Antibody-HRP is added, a complex of Antibody-HRP human IgA antibody virus N antigen forms on the microplate. A TMB substrate is added and a blue color is generated. The depth of color is relative to the amount of the anti-SARS-CoV-2 IgA antibody present. The Stop Solution changes the color from blue to yellow, and the intensity of the color is measured at 450 nm.

The Positive Control is from an inactivated serum sample which contains SARS-COV-2 N protein human IgA antibody. We do not know the exact amount of SARS-COV-2 N protein human IgA antibody in the Positive Control sample. The Positive Control can be used as calibration curve for interpretation purposes in different assays.

2. Protocol Summary

Prepare all reagents, samples, and positive controls as instructed.

Add 100 µl positive control, negative control, or sample to each well.
Incubate 1.5 hours at room temperature.

Add 100 µl prepared HRP- anti positive control IgA Antibody and/or
100 µl of prepared HRP-anti Human IgA Antibody to each well.
Incubate 1 hour at room temperature.

Add 100 µL TMB Substrate Solution to each well.

Incubate 10 minutes at room temperature.

Add 50 µL Stop Solution to each well.

Read at 450 nm immediately.

3. Precautions

Please read these instructions carefully prior to beginning the assay.

- All ELISA kit components have been formulated and quality control tested to function successfully as a kit.
- We understand that, occasionally, experimental protocols might need to be modified to meet unique experimental circumstances. However, we cannot guarantee the performance of the product outside the conditions detailed in this protocol booklet.
- Reagents should be treated as possible mutagens and should be handle with care and disposed of properly. Please review the Safety Datasheet (SDS) provided with the product for information on the specific components.
- Observe good laboratory practices. Gloves, lab coat, and protective eyewear should always be worn. Never pipette by mouth. Do not eat, drink or smoke in the laboratory areas.
- All biological materials should be treated as potentially hazardous and handled as such. They should be disposed of in accordance with established safety procedures.

4. Storage and Stability

The entire ELISA kit may be stored at -20°C for up to 1 year from the date of shipment. Avoid repeated freeze-thaw cycles. The kit may be stored at 4°C for up to 6 months. For extended storage, it is recommended to store at -80°C.

Observe the storage conditions for individual prepared components in the Reagent Preparation Section 9.

5. Limitations

- ELISA kit intended for research use only. Not for use in diagnostic procedures.
- Do not mix or substitute reagents or materials from other kit lots or vendors. Kits are QC tested as a set of components and performance cannot be guaranteed if utilized separately or substituted.

6. Materials Supplied

Item	Quantity	Storage Condition
HRP-anti IgA Antibody	1 vial	-80°C
20X Wash Buffer	25 mL	-80°C
5X Assay Diluent B	15 mL	-80°C
Stop Solution	8 mL	-80°C
TMB One-Step Substrate Solution	12 mL	-80°C
SARS-CoV-2 N protein coated Microplate	1 unit	-80°C
Positive Control	1 vial	-80°C
Negative Control	1vial	-80°C

7. Materials Required, Not Supplied

These materials are not included in the kit, but will be required to successfully perform this assay:

- Microplate reader capable of measuring absorbance at 450 nm.
- Precision pipettes to deliver 2 µl to 1 ml volumes.
- Adjustable 1-25 ml pipettes for reagent preparation.
- 100 ml and 1 liter graduated cylinders.
- Absorbent paper.
- Distilled or deionized water.
- Tubes to prepare positive control or sample dilutions.

8. Technical Hints

- Samples generating values higher than the highest positive control should be further diluted in the appropriate sample dilution buffers.
- Avoid foaming or bubbles when mixing or reconstituting components.
- Avoid cross contamination of samples or reagents by changing tips between sample, positive control and reagent additions.
- Ensure plates are properly sealed or covered during incubation steps.
- Complete removal of all solutions and buffers during wash steps is necessary to minimize background.
- All samples should be mixed thoroughly and gently.
- Avoid multiple freeze/thaw of samples.
- When generating positive control samples, it is advisable to change pipette tips after each step.
- This kit is sold based on number of tests. A 'test' simply refers to a single assay well. The number of wells that contain sample or positive control will vary by product. Review the protocol completely to confirm this kit meets your requirements. Please contact our Technical Support staff with any questions.

9. Reagent Preparation

- Equilibrate all reagents to room temperature (18-25°C) prior to use. The kit contains enough reagents for 96 wells.
- Prepare only as much reagent as is needed on the day of the experiment.

9.1 Assay Diluent B:

5X Assay Diluent B should first be diluted 5-fold with deionized water before use to make 1X Assay Diluent B.

9.2 Positive Control:

Add 400 µl of 1X Assay Diluent B into the Positive Control vial to prepare the Positive Control.

9.3 HRP-anti Human IgA Antibody:

Briefly spin vial. Add 150 µL of 1X Assay Diluent B into the vial to prepare a detection antibody concentrate. Pipette up and down to mix gently (the concentrate can be stored at 4°C for 5 days). The detection antibody concentrate should be diluted 100-fold with 1X Assay Diluent B.

9.4 20X Wash Buffer:

If the Wash Concentrate (20X) contains visible crystals, warm to room temperature and mix gently until dissolved. Dilute 20 mL of Wash Buffer Concentrate into deionized or distilled water to yield 400 mL of 1X Wash Buffer.

10. Sample Preparation

- Equilibrate all reagents to room temperature (18-25°C) prior to use. The kit contains enough reagents for 96 wells.
- Prepare only as much sample as is needed on the day of the experiment.

10.1 Human serum or plasma:

Dilute sample (human serum or plasma) with 1X Assay Diluent B 500 times.

For example, add 0.5µl serum + 249.5µl Assay Diluent B. Mix the diluted sample well and evenly for the best results.

Δ Note: The user needs to calculate the amount of the sample used for the whole test. Please reserve sufficient amount of sample in advance.

Δ Note: Avoid using samples with severe hemolysis, precipitate, or contamination by bacteria or protein suspension.

Δ Note: The use of EDTA, heparin sulfate, sodium citrate, or other anticoagulants will not affect the results.

11. Assay Procedure

- Equilibrate all materials and prepared reagents to room temperature prior to use.
- We recommend that you assay all positive controls, negative controls and samples in duplicate.
- Prepare all reagents, working positive and negative controls, and samples as directed in the previous sections.

11.1 Label removable 8-well strips as appropriate for your experiment.

11.2 Add 100 µl of each positive control, negative control and sample (prepared in Reagent Preparation section) into appropriate wells. Cover wells and incubate for 1.5 hours at room temperature with gentle shaking.

11.3 Discard the solution and wash 4 times with 1X Wash Solution. Wash by filling each well with Wash Buffer (300 µL) using a multi-channel Pipette or auto-washer. Complete removal of liquid at each step is essential for 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.

11.4 Add 100 µl of prepared HRP-anti IgA Antibody to each well. Incubate for 1 hour at room temperature with gentle shaking.

11.5 Discard the solution. Repeat the wash as in step 11.3.

11.6 Add 100 µL of TMB Substrate Solution to each well. Incubate for 10 minutes at room temperature in the dark with gentle shaking.

11.7 Add 50 µL of Stop Solution to each well. Read at 450 nm immediately.

12. Interpretation of Results

12.1 Although a negative control is provided in the kit, it is recommended for the researcher to include at least one normal human serum/plasma sample as an additional negative control.

12.2 For the assay to be valid, the following specifications must be met: (1) the Positive Control mean optical density (PC: OD450) must be greater than 0.5. (2) the negative control(s) mean should be less than 0.3.

12.3 A positive result in an unknown sample is defined as an average OD which is 2 standard deviations above the negative control. If the researcher has provided their own negative control, they should use it in this calculation instead of the Negative Control supplied in kit.

12.4 The SARS-CoV-2 N protein coated in this kit is a recombinant protein expressed by mammalian cells. The antibodies used in this assay are polyclonal. The positive control in this kit is an inactivated serum sample containing SARS-CoV-2 N protein IgA antibody.

12.5 This kit is not suitable for samples containing sodium azide (NaN3) which will affect the reactivity of HRP and result in the underestimation of the SARS-CoV-2 IgA Antibody levels.

13. Troubleshooting

Problem	Reason	Solution
Poor positive control curve	Inaccurate Pipetting	Check pipettes
	Improper positive control dilution	Prior to opening, briefly spin the stock positive control tube and dissolve the powder thoroughly by gentle mixing
Low Signal	Improper preparation of positive control and/or HRP antibody	Briefly spin down vials before opening. Dissolve the powder thoroughly.
	Too brief incubation times	Ensure sufficient incubation time. Sample and positive control addition may be done overnight at 4°C with gentle shaking (note: may increase overall signals including background).
	Inadequate reagent volumes or improper dilution	Check pipettes and ensure correct preparation
Large CV	Inaccurate pipetting	Check pipettes
	Air bubbles in wells	Remove bubbles in wells
High background	Plate is insufficiently washed	Review the manual for proper wash. If using a plate washer, ensure that all ports are unobstructed.
	Contaminated wash buffer	Make fresh wash buffer
Low sensitivity	Improper storage of the ELISA kit	Store your positive control at <-70°C after reconstitution, others at 4°C. Keep substrate solution protected from light.
	Stop solution	Add stop solution to each well before reading plate

14. Notes

Technical Support

For all technical or commercial enquiries please go to:

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