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ab274339
COVID-19 N-Protein Human IgG ELISA Kit

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

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

Table of Contents

1. Overview

COVID-19 N-Protein Human IgG ELISA Kit (ab274339) is an in vitro indirect ELISA for the quantitative measurement of human IgG antibody against SARS-CoV-2 N protein in human serum.

Standard 96-well plates (12 strips with 8 wells/strip) are coated with the SARS-CoV-2 N protein, which combines with the corresponding antibody present in a sample and Positive Control which used as calibration curve for interpretation purposes. The wells are washed, and biotinylated anti-human IgG antibody is added. After washing away unbound biotinylated antibody, HRP-conjugated streptavidin is pipetted to the wells. The wells are again washed, a TMB substrate solution is added to the wells and color develops in proportion to the amount of COVID19 N protein human IgG antibody bound. The Stop Solution changes the color from blue to yellow, and the intensity of the color is measured at 450 nm.

The Positive Controls are from an inactivated serum sample which contains SARS-COV-2 N protein human IgG antibody. We do not know the exact amount of SARS-COV-2 N protein human IgG 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 or sample to each well.
Incubate 1 hour at room temperature.

Add 100 µL prepared biotin antibody to each well.
Incubate 30 minutes at room temperature.

Add 100 µL prepared Streptavidin solution.
Incubate 30 minutes at room temperature.

Add 100 µL TMB Substrate Solution to each well.
Incubate 15 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. 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
SARS-CoV-2 N protein coated Microplate	1 unit	-20°C
20X Wash Buffer	25 mL	-20°C
Positive Control	1 vial	-20°C
Biotinylated anti-Human IgG Antibody	2 vials	-20°C
HRP-Streptavidin Concentrate	1 vial	-20°C
TMB One-Step Substrate Reagent	12 mL	-20°C
Stop Solution	8 mL	-20°C
5X Assay Diluent B	15 mL	-20°C
5X Sample Diluent	25 mL	-20°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 standard 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, control 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 5X Assay Diluent B:

Dilute 5X Assay Diluent B 5-fold with deionized or distilled water before use.

9.2 5X Sample Diluent:

Dilute 5X Sample Diluent 5-fold with deionized or distilled water before use.

9.3 Biotinylated anti-Human IgG Antibody:

Briefly spin. Add 200 µL of 1X Assay Diluent B into the vial to prepare an 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.

9.5 HRP-Streptavidin Concentrate:

Briefly spin the vial of HRP-Streptavidin concentrate before use. HRP-Streptavidin should be diluted 800-fold with 1X Assay Diluent B.

For example: Briefly spin the vial and pipette up and down to mix gently. Add 10 µL of HRP-Streptavidin concentrate into a tube with 8 mL 1X Assay Diluent B to prepare an 800-fold diluted HRP-Streptavidin solution (don't store the diluted solution for next day use). Mix well.

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:

Dilute sample with 1X Sample Diluent (prepared in step 9.2) 1500 times. Mix the diluted sample well and evenly for the best results.
For example, add 1 µL serum + 1499 µL 1X Sample Diluent.

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, 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. Positive Control Preparation

- Always prepare a fresh set of Positive Controls for every use.
- Discard working dilutions after use as they do not store well.
- The following section describes the preparation of a calibration curve for duplicate measurements (recommended).

11.1 Briefly spin the vial of Positive Control.
11.2 Add 400 µL 1X Sample Diluent into the vial to prepare a 1000 Unit/ml Positive Control solution. Dissolve the powder thoroughly by a gentle mix.
11.3 Pipette 320 µL 1X Sample Diluent into each of 7 tubes.
11.4 Use the 1000 Unit/ml Positive Control solution to produce a dilution series (shown below). Mix each tube thoroughly before the next transfer.
11.5 1X Sample Diluent serves as the zero (0 Unit/ml).

Tube #	Volume to dilute	Volume of 1X Sample Diluent	Final Concentration Unit/mL
1	1000 U/mL Positive Control Solution	---	1000
2	160 µL of tube #1	320 µL	333.3
3	160 µL of tube #2	320 µL	111.1
4	160 µL of tube #3	320 µL	37.04
5	160 µL of tube #4	320 µL	12.35
6	160 µL of tube #5	320 µL	4.12
7	160 µL of tube #6	320 µL	1.37
8	---	320 µL	0

12. Assay Procedure

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

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

12.2 Add 100 µL of prepared Positive Control and sample into appropriate wells. Cover the wells and incubate for 1 hour at room temperature with gentle shaking.

12.3 Discard the solution and wash 4 times with 1X Wash Buffer. 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.

12.4 Add 100 µL of 1X prepared biotinylated antibody to each well. Incubate for 30 minutes at room temperature with gentle shaking.

12.5 Discard the solution. Repeat the wash as in step 12.3.

12.6 Add 100 µL of prepared Streptavidin solution each well. Incubate for 30 minutes at room temperature with gentle shaking.

12.7 Discard the solution. Repeat the wash as in step 12.3.

12.8 Add 100 µL of TMB One-Step Substrate Solution to each well. Incubate for 15 minutes at room temperature in the dark with gentle shaking.

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

13. Calculations

13.1 Calculate the mean absorbance for each set of duplicate positive controls and samples.

13.2 Subtract the average zero positive control optical density.

13.3 Plot the calibration curve on log-log, with positive control concentration (U/mL) on the x-axis and absorbance on the y-axis using Sigma plot or excel software.

13.4 A calibration curve must be run with each assay.

14. Typical Data

Typical calibration curve – data provided **for demonstration purposes only**. A new calibration curve must be generated for each assay performed.

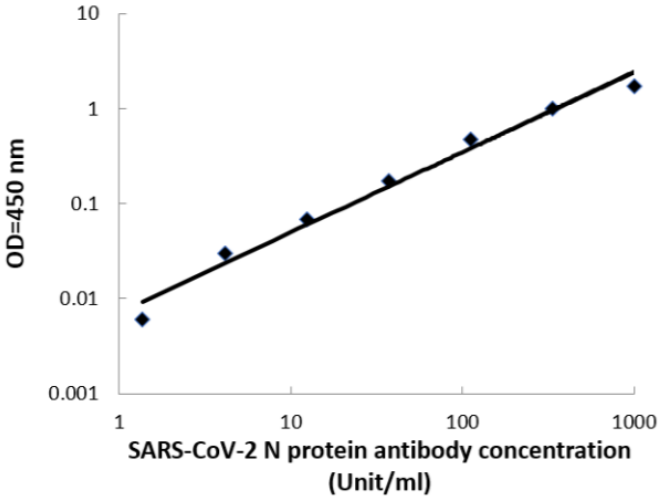

Figure 1. COVID-19 N-Protein Human IgG ELISA kit (ab274339) calibration curve.

15. Troubleshooting

Problem	Reason	Solution
Poor calibration curve	Inaccurate Pipetting	Check pipettes
	Improper control dilution	Prior to opening, briefly spin the positive control tube and dissolve the powder thoroughly by gentle mixing
Low Signal	Improper preparation of control and/or biotinylated antibody	Briefly spin down vials before opening. Dissolve the powder thoroughly.
	Too brief incubation times	Ensure sufficient incubation time. Sample and 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 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

16. Notes

Technical Support

For all technical or commercial enquiries please go to:

www.abcam.com/contactus

www.abcam.cn/contactus (China)

www.abcam.co.jp/contactus (Japan)