antibodies-online™

ABIN6952781

Sandwich ELISA Kit for SARS-CoV-2 N-Protein Antibody

Human,SARS Coronavirus-2 (SARS-CoV-2)

96 tests

For research use only
Not for use in clinical diagnostic procedures
Version Aug 2022

Intended use

anti-2019-nCoV IgG ELISA Kit allows for the in vitro qualitative determination of 2019 nCoV-Ig antibody in serum, plasma and saliva and nasal fluid.

Reagents and materials provided

- Coated assay plate
- Negative Control (Ready-to-use)
- Positive Control (Ready-to-use)
- Sample Dilution Buffer
- Biotin-conjugated Nucleocapsid (Concentrated)
- Antigen Dilution Buffer
- HRP-Streptavidin Conjugate(SABC)
- SABC Dilution Buffer
- Wash Buffer (25 x concentrate)
- TMB Substrate
- Stop solution
- Plate Sealer
- Product Description

Materials required but not supplied

Storage of the kit

2-8°C for 6 months

Note:

It is strongly recommended to use the remaining reagents within 1 month, if this is done before the expiry date of the kit. Please refer to the label on the kit packaging for the expiration date of the kit. All components are stable until the expiration date.

Sample collection and storage

Sample type	Collection procedure
Plasma	Collect plasma using EDTA or heparin as an anticoagulant. Centrifuge samples for 15 min at 1000×g at 2-8°C within 30 min of collection. Collect the supernatant and assay immediately or store samples in aliquot at -20°C or -80°C for later use. Avoid repeated freeze/thaw cycles. Hemolysed samples are not suitable for ELISA assay!
Saliva	Collect saliva using a collection device or equivalent. Remove particulates by centrifugation for 15 minutes at 1000×g at 2-8°C. Collect the supernatant and assay immediately or store samples in aliquot at ≤-20°C. Avoid repeated freeze/thaw cycles. It is recommended to use fresh saliva samples.
Serum	Use a serum separator tube and allow samples to clot for 2 hours at room temperature or overnight at 4°C before centrifugation for 20 min at 1000×g. Collect the supernatant and assay freshly prepared serum immediately or store samples in aliquot at -20°C or -80°C for later use. Avoid repeated freeze/thaw cycles.

Note:

1. Samples to be used within 5 days may be stored at 4 °C, otherwise samples must be stored at -20 °C (≤ 1 month) or -80 °C (≤ 2 months) to avoid loss of bioactivity and contamination.
2. Sample hemolysis will influence the result, so hemolytic specimen should not be used.
3. When performing the assay, bring samples to room temperature.

Reagent preparation

Wash Buffer Preparation:
If crystals have formed in the concentrate, you can warm it with 40°C water bath (Heating temperature should not exceed 50°C) and mix it gently until the crystals have completely been dissolved. The solution should be cooled to room temperature before use.
Dilute 30ml Concentrated Wash Buffer into 750ml Wash Buffer with deionized or distilled water. Put unused solution back at 2-8°C.
Preparation of HRP-conjugated anti-human IgG Working Solution:
Prepare it within 1 hour before experiment.

- Calculate required total volume of the working solution: 50ul / well × quantity of wells. (Allow 55-60ul more than the total volume.)

- Dilute the HRP-conjugated anti-human IgG with Antibody Dilution Buffer at 1:100 and mix them thoroughly. (i.e. Add 1µl HRP-conjugated anti-human IgG into 99µl Antibody Dilution Buffer.)

Sample preparation

- It is recommended to use fresh samples without long storage, otherwise protein degradation and denaturationmay occur in these samples, leading to false results. Samples should therefore be stored for a short periodat 2 - 8 °C or aliquoted at -20 °C (≤1 month) or -80 °C (≤ 3 months). Repeated freeze-thawcycles should be avoided. Prior to assay, the frozen samples should be slowly thawed and centrifuged toremove precipitates.
- If the sample type is not specified in the instructions, a preliminary test is necessary to determinecompatibility with the kit.
- If a lysis buffer is used to prepare tissue homogenates or cell culture supernatant, there is a possibilityof causing a deviation due to the introduced chemical substance.The recommended dilution factor is for reference only.
- Please estimate the concentration of the samples before performing the test. If the values are not in therange of the standard curve, the optimal sample dilution for the particular experiment has to be determined.Samples should then be diluted with PBS (pH =7.0-7.2).

Note:

Isolate the test samples soon after collecting, then, analyze immediately (within 2 hours). Or aliquot and store at -20℃ for long term. Avoid multiple freeze-thaw cycles.

- Serum: Coagulate the serum at room temperature (about 1 hour). Centrifuge at approximately 1000 × g for 15 min. Analyze the serum immediately or aliquot and store at -20℃.
- Plasma: Collect plasma with heparin or EDTA as the anticoagulant. Centrifuge for 15min at 2-8°C at 1500 x g within 30 min of collection. For eliminating the platelet effect, suggesting that further centrifugation for 10 min at 2-8°C at 10000 x g. Analyze immediately or aliquot and store frozen at -20℃.
- Saliva & Nasal fluid: Centrifuge samples for 20 minutes at 10000×g at 2-8°C. Collect supernatant and carry out the assay immediately.

Assay procedure

AssayProcedure: When diluting samples and reagents, they must be mixed completely and evenly. Before adding TMB into wells, equilibrate TMB Substrate for 30 min at 37 °C.

1. Bring all reagents to room temperature before use.
2. Label the sample wells, 2 Negative Controls, 2 Positive Controls and 1 blank well.Wash plate 2 times before adding sample and control (blank) wells!
3. Add 45 µL sample dilution buffer to each sample well. Add 50 µL sample dilution buffer for blank well.
4. Add 5µL sample to each sample well. Add 50ul Negative Controls and Positive Controls to set Controls well and gently tap the plate to ensure thorough mixing. Seal the plate with a cover and incubate at 37**℃ for 30 min.**
5. Remove the cover, and wash plate 2 times with Wash buffer and each time let the wash buffer stay in the wells for 1-2 min.
6. Add 50 µLBiotin-labeled Antigento each well.Seal the plate with a cover and incubate at 37**℃ for 30 min.**
7. Remove the cover, and wash plate 3 times with Wash buffer and each time let the wash buffer stay in the wells for 1-2 min.
8. Add 50µl of SABC Working Solution into each well, cover the plate and incubate at 37°C for 30 minutes.
9. Remove the cover, and wash plate 5 times with Wash buffer and each time let the wash buffer stay in the wells for 1-2 min.
10. Add 50µl of TMB substrate into each well. Gently tap the plate to ensure thorough mixing. Cover the plate and incubate at 37**℃ in dark within 10-20 min. And the shades of obvious blue can be seen in the Positive Controls. Blank well wells show no obvious color.**
11. Add 50 µl of Stop solution into each well and mix thoroughly. The color changes into yellow immediately.
12. Read the O.D. absorbance at 450 nm in a microplate reader immediately after adding the stop solution(Use the blank well to set zero).

Test principle

This kit was based on sandwich enzyme-linked immune-sorbent assay technology. Capture antibody was pre-coated onto 96- well plates. And the biotin conjugated antibody was used as detection antibodies. The standards, test samples and biotin conjugated detection antibody were added to the wells subsequently, and washed with wash buffer. HRP-Streptavidin was added and unbound conjugates were washed away with wash buffer. TMB substrates were used to visualize HRP enzymatic reaction. TMB was catalyzed by HRP to produce a blue color product that changed into yellow after adding acidic stop solution. The density of yellow is proportional to the target amount of sample captured in plate. Read the O.D. absorbance at 450nm in a microplate reader, and then the

concentration of target can be calculated.

Calculation of results

Cutoff Value =NCx × 2.1
NCx: Mean Absorbance of Negative Control (when NCx<0.05, Calculate as 0.05).
PCx : Mean Absorbance of Positive Control
1. Sample with absorbance values < Cutoff Value are considered negative.
Sample with absorbance value ≥ Cutoff Value are considered positive.
2. PCx≤0.5, the test is regarded as Invalid, should be tested again.

Typical data

In order to make the calculation easier, we plot the O.D. value of the standard (X-axis) against the log of concentration of the standard (Y-axis), although concentration is the independent variable and O.D. value is the dependent variable. The O.D. values of the standard curve may vary according to the conditions of assay performance (e.g. operator, pipetting technique, washing technique or temperature effects).

Detection range

The detection range of the kit is

Stability

The stability of ELISA kit is determined by the loss rate of activity. The loss rate of this kit is less than 5 % prior to the expiration date under appropriate storage condition. To minimize extra influence on the performance, operation procedures and lab conditions, especially room temperature, air humidity, incubator temperature should be strictly monitored. It is also strongly suggested that the assay is performed by the same operator from the beginning to the end.

Assay procedure summary

This kit was based on sandwich enzyme-linked immune-sorbent assay technology. Recombinant 2019 nCoV Nucleocapsid protein (antigen) was pre-coated onto 96-well plates. The Controls, test samples and Biotin-labeled antigen were added to the wells subsequently, and wash with wash buffer. HRP-Streptavidin Conjugate was added and unbound conjugates were washed away with wash buffer. TMB was catalyzed by HRP to produce a blue color product that changed into yellow after adding acidic stop solution. The optical density of developed color is read with a suitable photometer at 450nm with a selected reference wavelength within 650 nm.

Important note

1. The kit is designed for research use only, we will not be responsible for any issue if the kit was used in clinical diagnostic or any other procedures.
2. Limited by the current conditions and scientific technology, we cannot perform a complete identification and analysis of the raw material used. Therefore, the use of the kit may be associated with some qualitative and technical risks.
3. We are only responsible for the kit itself, not for the samples used in the test. The possible amount of sample used in the whole test should be calculated in advance and sufficient sample material should be provided.
4. Each kit undergoes a very strict QC testing. Nevertheless, end-user results may differ from our internal results due to unexpected transport conditions or different laboratory equipment. Intra-assay deviations between kits from different lots can also be related to this.
5. The test results depend on the validity of the products, so the kit should be used before the expiration date and stored according to the instructions.
6. Even the same user may obtain different results in two separate experiments. To obtain reproducible results, each step of the assay should be controlled.
7. The standard of the kit and immunogen used for antibody preparation are commonly recombinant proteins, as different fragments, expression systems, purification methods might be used in recombinant protein preparation, we can not guarantee the kit could detect recombinant protein from other companies. So, it is not recommended to use the kit for the detection of recombinant protein.
8. Do not mix or substitute reagents from one kit lot to another. Use only the reagents supplied by the manufacturer.
9. Protect all reagents from strong light during storage and incubation. All reagent bottle caps should be tightly closed to prevent evaporation and contamination with microorganisms. The TMB substrate should remain colourless until it reacts with the enzyme.
10. A freshly opened ELISA plate may show a water-like substance, which is normal and does not affect the test results. Return unused wells to the foil pouch and store as before.
11. Mistakes in reagent preparation and application, as well as incorrect parameter setting for the plate reader, can lead to incorrect results. A microplate reader with a bandwidth of 10nm or less and an optical density range of 0-3 O.D. at a wavelength of 450 ± 10 nm is suitable for absorbance measurement. Please read the instructions carefully and set up the instrument before the experiment.

12. Do not reuse the reconstituted standard and the prepared working solutions. The unused stock solutions should be stored according to the storage conditions.

Precaution

The Stop Solution suggested for use with this kit is an acid solution. Wear eye, hand, face, and clothing protection when using this material.

Troubleshooting

Problem	Possible Source	Corrective Action
Poor Standard Curve	Improper standard curve preparation	Ensure accurate operation of the dilution
	Incomplete washing and aspiration	Adequate washing and adequate aspiration
	Inaccurate Pipetting	Check and Calibrate pipettes
Poor Precision	Incomplete washing of wells	Ensure sufficient washing
	Inadequate mixing and aspiration reagents	Adequate aspiration and mixing reagents
	Reused pipette tips, containers and sealers	Change and use new pipette tips, containers and sealers
	Inaccurate Pipetting	Check and Calibrate pipettes
Low O.D Values	Inadequate reagent volumes added to wells	Calibrate pipettes and add adequate reagents
	Incorrect incubation times	Ensure sufficient incubation times
	Incorrect incubation temperature	Reagents balanced to room temperature
	Conjugate or substrate reagent failure	Mix conjugate and substrate, color should develop immediately
	No stop solution added	Follow the assay protocol in the kit manual
	Read beyond suggested reading time	Read within the time recommended in the manual

Problem	Possible Source	Corrective Action
Sample Values	Improper Sample Storage	Store the sample properly and use the fresh sample
	Improper sample collection and preparation	Take proper sample collection and preparation method
	Low quantity of analyte in samples	Resample and repeat assay

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For more information, please contact:

antibodies-online Inc.
PO Box 5201
Limerick, PA 19468
USA

Website: www.antibodies-online.com
Email: info@antibodies-online.com
Phone: +1 877 302 8632
Fax: +1 888 205 9894

antibodies-online GmbH
Schloss-Rahe-Straße 15
52072 Aachen
Deutschland

Website: www.antikoerper-online.de
Email: info@antikoerper-online.de
Phone: +49 (0)241 95 163 153
Fax: +49 (0)241 95 163 155