SARS-CoV-2 Total Antibody ELISA Kit

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Read the package insert carefully and completely before performing the assay. Follow the instructions and do not modify them. Only by strict adherence to these instructions, the erroneous results can be avoided, and the optimal performance of SARS-CoV-2 Total Antibody ELISA Kit achieved.

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Intended Use

SARS-CoV-2 Total Antibody ELISA Kit is an enzyme-linked immunosorbent assay intend for qualitative detection of total antibodies to SARS-CoV-2 virus in human serum or plasma specimens. This kit is for research use only. It is not for use in the diagnosis or screening of COVID-19.

General Description

COVID-19, formerly known as SARS-CoV-2, is an enveloped virus that contains a single-stranded positive-sense RNA. Common signs of infection include respiratory symptoms, fever, and cough, shortness of breath and breathing difficulties. In severe cases, infection can cause pneumonia, severe acute respiratory syndrome (SARS), kidney failure and death. SARS-CoV-2 Total Antibody ELISA Kit detects IgM, IgA, and IgG antibodies to SARS-CoV-2. In conjunction with other diagnostic tests it can be used to determine if an individual has been infected with SARS-CoV-2.

Principles of Testing

SARS-CoV-2 Total Antibody ELISA Kit is a two-step incubation antigen "sandwich" enzyme immunoassay kit, which uses polystyrene microwell strips pre-coated with recombinant SARS-CoV-2 antigen. The tested serum or plasma specimen is added to pre-coated microwell, and during the first incubation, the specific SARS-CoV-2 antibodies will be captured inside the wells if present. The microwells are then washed to remove unbound serum proteins. Second recombinant SARS-CoV-2 antigen conjugated to the Enzyme Horseradish Peroxidase (HRP-Conjugate) is added, and during the second incubation, the conjugated antigen will bind to the captured antibody inside the wells. The microwells are then washed to remove unbound conjugate, and Chromogen Solutions are added into the wells. In wells containing the antigen-antibody-antigen (HRP) "sandwich" immunocomplex, the colorless TMB are hydrolyzed by the bound HRP conjugate to a blue colored product. The blue color turns yellow after the reaction is stopped with sulfuric acid. The amount of color intensity can be measured and it is proportional to the amount of antibody captured inside the wells, and to the specimen respectively. Wells containing specimens negative for SARS-CoV-2 antibodies remain colorless.

Reagents And Materials Provided

1. **Microwell Plate (12×8-wells):** pre-coated microwell strips fixed on white strip holder. The plate is sealed in aluminum pouch with desiccant. Each well contains recombinant SARS-CoV-2 antigen. The microwell strips can be used separately. Place unused wells or strips in the provided plastic sealable storage bag together with the desiccant and return to 2-8°C. Once opened, stable for 2 weeks at 2-8°C.
2. **Negative Control (1.0 mL):** The liquid filled in a 1.5 ml vial. Protein-stabilized buffer tested non-reactive for SARS-CoV-2 antibodies. Ready to use as supplied. Once opened, stable for 2 weeks at 2-8°C.
3. **Positive Control (0.8 mL):** The liquid filled in a 1.5ml vial. SARS-CoV-2 positive material diluted in protein-stabilized buffer. Ready to use as supplied. Once opened, stable for 2 weeks at 2-8°C.
4. **100 × HRP-Enzyme Conjugate (130 μL):** 100 × HRP-labeled recombinant SARS-CoV-2 antigen enzyme conjugate filled in a 0.5 ml vial. Once opened, stable for 2 weeks at 2-8°C.
5. **HRP-Enzyme Conjugate Diluent (12 mL):** HRP-conjugate diluent filled in a 15ml white bottle. The 120 μL of 100× HRP-Enzyme Conjugate is diluted directly into 12 mL HRP-Enzyme Conjugate Diluent. After diluting, the 1×HRP-enzyme conjugate solution can be stored up to 1 week at 2-8°C.
6. **20X Wash Buffer (50 mL):** 20X Wash Buffer filled in a white bottle. Buffer solution containing surfactant. The concentrate must be diluted 1 to 20 with deionized water before use. Once diluted, stable for 1 week at room temperature, or for 2 weeks when stored at 2-8°C.
7. **TMB Solution (12 mL):** TMB Solution filled in a brown bottle. This solution contains 3, 3', 5, 5'-tetramethylbenzidine and hydrogen peroxide in a citric acid-citrate buffer (pH 3.3-3.8). Once opened, stable for 2 weeks at 2-8°C.
8. **Sample Diluent (12 mL):** Sample Diluent buffer filled in a 15 mL white bottle. This buffer is used to dilute the serum or plasma specimens. Once opened, stable for 2 weeks at 2-8°C.
9. **Stop Solution (6 mL):** Stop solution filled in a white bottle. Diluted sulfuric acid solution (2 M H_2SO4). Ready to use as supplied. Once opened, stable for 2 weeks at 2-8°C.
10. **Plastic Sealable Bag (2 unit):** For enclosing the strips not in use.
11. **Package Insert (1 copy)**
12. **Cardboard plate cover (2 sheets)**

Materials Required But Not Supplied

1. Freshly distilled or deionized water
2. Disposable gloves
3. Timer
4. Appropriate waste containers for potentially contaminated materials
5. Dispensing system and/or pipette, disposable pipette tips
6. Absorbent tissue or clean towel
7. Dry incubator or water bath, 37±1°C
8. Plate reader, single wavelength 450nm or dual wavelength 450/600~650nm
9. Microwell aspiration/wash system

Storage

The components of the kit will remain stable through the expiration date indicated on the label and package when stored between 2-8°C, do not freeze. To assure maximum performance of SARS-CoV-2 Total Antibody ELISA Kit, during storage, protect the reagents from light, microorganism and chemicals.

Specimen Collection And Preparation

1. **Specimen Collection:** No special patient's preparation required. Collect the specimen in accordance with the normal laboratory practice. Either fresh serum or plasma specimens can be used with this assay. Blood collected by venipuncture should be allowed to clot naturally and completely – the serum/plasma must be separated from the clot as early as possible as to avoid haemolysis of the RBC. Care should be taken to ensure that the serum specimens are clear and not contaminated by microorganisms.
2. Plasma specimens collected into EDTA, sodium citrate or heparin can be tested, but highly lipaemic, icteric, or hemolytic specimens should not be used as they can give false results in the assay**. Do not heats inactivate specimens.** This can cause deterioration of the target analyte. Specimens with visible microbial contamination should never be used.
3. SARS-CoV-2 Antibody ELISA is intended ONLY for testing of individual serum or plasma specimens. Do not use the assay for testing of cadaver specimens, saliva, urine or other body fluids, or pooled (mixed) blood.
4. **Transportation and Storage:** Store specimens at 2-8°C. Specimens not required for assaying within 1 week should be stored frozen (-20°C or lower). Multiple freeze-thaw cycles should be avoided. For shipment, specimens should be packaged and labeled in accordance with the existing local and international regulations for transportation of clinical specimens and ethological agents.

Reagents Preparation

1. Allow the reagents and tested serum or plasma to reach room temperature (18-30°C).
2. Check the Wash buffer concentrate for the presence of salt crystals. If crystals have formed, resolubilize by warming at 37°C until crystals dissolve. Dilute the Wash buffer (20X) as indicated in the instructions for washing. Use distilled or deionized water and only cleans vessels to dilute the buffer.
3. Add 120 µL of 100× HRP-Enzyme Conjugate directly to the 12 mL HRP-Enzyme Conjugate Diluent. After diluting, the 1×HRP-enzyme conjugate solution can be stored up to 1 week at 2-8°C. After 1 week, this conjugate solution should be discarded and no longer used in this assay. All other reagents are **READY TO USE AS SUPPLIED.**

Assay Procedure

1. **Preparation:** Mark three wells as Negative control (e.g. B1, C1, D1), two wells as Positive control (e.g. E1, F1) and one Blank (e.g. A1, neither specimens nor HRP-Conjugate should be added into the Blank well). If the results will be determined by using dual wavelength plate reader, the requirement for use of Blank well could be omitted. Use only number of strips required for the test.
2. **Adding Controls and Specimen:** Add 100 µL of Positive control, Negative control. Add 50 µL of Sample Diluent in each sample detection well of the microplate and add 50 µL of serum or plasma specimen into their respective wells except the Blank. Note: Use a separate disposal pipette tip for each specimen, Negative Control, Positive Control to avoid cross-contamination.
3. **Incubating:** Cover the plate with the plate cover and incubate at 37°C for 60 minutes.
4. **Washing:** At the end of the incubation, remove and discard the plate cover. Wash each well 5 times with 300µL diluted Wash Buffer. Each time allow the microwells to soak for 30-60 seconds. After the final washing cycle, turn down the plate onto blotting paper or clean towel, and tap it to remove any remainders.
5. **Adding 1X HRP-Conjugate:** Prepare the 1X HRP Conjugate Solution (add 120 µL of 100× HRP-Enzyme Conjugate directly into 12 mL conjugate diluent solution) and add 100 µL per well of 1X HRP Conjugate Solution into each well except the Blank.
6. **Incubating:** Cover the plate with the plate cover and incubate at 37°C for 30 minutes.
7. **Washing:** At the end of the incubation, remove and discard the plate cover. Wash each well 5 times with 300µL diluted Wash Buffer. Each time allow the microwells to soak for 30-60 seconds. After the final washing cycle, turn down the plate onto blotting paper or clean towel, and tap it to remove any remainders.
8. **Coloring:** Add 100 µL TMB Solution into each well including the Blank. Incubate the plate at 37°C for 30 minutes avoiding light. The enzymatic reaction between the TMB solution and the HRP-

Conjugate produces blue color in Positive control and SARS-CoV-2 antibody positive specimen wells.

9. **Stopping Reaction:** Using a multichannel pipette or manually, add 50 µL of Stop Solution into each well and mix gently. Intensive yellow color develops in Positive control and SARS-CoV-2 antibody positive specimen wells.
10. **Measuring the Absorbance:** Calibrate the plate reader with the Blank well and read the absorbance at 450nm and set the reference wavelength at 600~650nm. Calculate the Cut-off value and evaluate the results. (Note: read the absorbance within 10 minutes after stopping the reaction).

Calculation

Each microplate should be considered separately when calculating and interpreting the results of the assay, regardless of the number of plates concurrently processed. The results are calculated by relating each specimen absorbance (A) value to the Cut-off value (C.O.) of the plate. If the Cut-off reading is based on single filter plate reader, the results should be calculated by subtracting the Blank well's A value from the print report values of specimens and controls. In case the reading is based on dual filter plate reader, do not subtract the Blank well's A value from the print report values of specimens and controls.

Calculation of the Cut-off value (C.O.) = 0.10 + Nc

(Nc = the mean absorbance value for three negative controls). If Nc is < 0.05, take it as 0.05.

Quality Control

The test results are valid if the Quality Control criteria are fulfilled. It is recommended that each laboratory must establish appropriate quality control system with quality control material similar to or identical with the patient specimen being analyzed.

- **The absorbance (A) values of the Positive control wells must be ≥ 0.40 at 450/600~650 nm or at 450 nm after subtracting blank.**
- **The absorbance (A) values of the Negative control wells at least two values must be < 0.10 at 450/600~650 nm or at 450 nm after subtracting blank.**

If one of the Negative control absorbance (A) value does not meet the Quality Control criteria, it should be discarded and the mean value calculated again using the remaining two values. If more than one Negative control A value do not meet the Quality Control Range specifications, the test is invalid and must be repeated.

Interpretation Of Results

Negative Results (A / C.O. < 1): Specimens giving absorbance less than the Cut-off value are negative for this assay, which indicates that no SARS-CoV-2 antibodies have been detected with SARS-CoV-2 Ab ELISA, therefore there are no serological indications for current or past coronavirus disease COVID-19.

Positive Results (A / C.O. ≥ 1): Specimens giving an absorbance equal to or greater than the Cut-off value are considered initially reactive, which indicates that SARS-CoV-2 antibodies have probably been detected using SARS-CoV-2 Ab ELISA. All initially reactive specimens should be retested in duplicate using SARS-CoV-2 Ab ELISA before the final assay results interpretation. Repeatedly reactive specimens can be considered positive for antibodies to SARS-CoV-2 therefore there are serological indications for current or past coronavirus disease COVID-19.

Borderline (A / C.O. = 0.9-1.1): Specimens with absorbance to Cut-off ratio between 0.9 and 1.1 are considered borderline and retesting of these specimens in duplicate is required to confirm the initial results.

- If, after retesting of the initially reactive specimens, both wells are negative results (A/C.O.<1.0), these specimens should be considered as non-repeatable positive (or false positive) and recorded as negative. As with many very sensitive ELISA assays, false positive results can occur due to the several reasons, most of which are connected with, but not limited to, inadequate washing step.
- If after retesting in duplicate, both wells are positive results (A / C.O. ≥ 1.0), the final result from this ELISA test should be recorded as repeatedly reactive. Repeatedly reactive specimens could be considered positive for antibodies to SARS-CoV-2 and therefore the patient is probably infected with the virus.
- If after retesting in duplicate, one well is positive result (A / C.O. ≥ 1.0) but one well is negative result (A/C.O.<1.0), Specimen is EQUIVOCAL, the patient should be followed up and collected another specimen for testing.

Performance Characteristics

Sensitivity and Specificity

Validation study of the SARS-CoV-2 Total Antibody ELISA Kit was conducted with 76 specimens from confirmed COVID-19 patients and 128 specimens from healthy individuals. The kit demonstrated the sensitivity of 94.7% (72/76) and the specificity of 98.4% (126/128).

Endogenous Interference Substances Studies

The study was performed with potentially endogenous interfering substances to determine the effect on results with the COVID-19 ELISA test kit. The hemoglobin may cause false positive. So in real serum or plasma testing, the hemolysis sample should be excluded.

No interference was seen with the following substances in negative, weakly positive, or moderately positive samples at the concentrations listed.

Interference Substances	Testing concentration
Cholesterol	4 mg/mL
Triacylglycerol	2.2 mg/mL
Bilirubin	0.4 mg/mL

Precautions

1. Do not exchange reagents from different lots or use reagents from other commercially available kits. The components of the kit are precisely matched for optimal performance of the tests.
2. Make sure that all reagents are within the validity indicated on the kit box and of the same lot. Never use reagents beyond their expiry date stated on labels or boxes.
3. Allow the reagents and specimens to reach room temperature (18-30°C) before use. Shake reagent gently before use. Return at 2-8°C immediately after use.
4. Use only sufficient volume of specimen as indicated in the procedure steps. Failure to do so, may cause low sensitivity of the assay.
5. Do not touch the exterior bottom of the wells; fingerprints or scratches may interfere with the reading. When reading the results, ensure that the plate bottom is dry and there are no air bubbles inside the wells.
6. Never allow the microplate wells to dry after the washing step. Immediately proceed to the next step. Avoid the formation of air bubbles when adding the reagents.
7. Avoid long time interruptions of assay steps. Assure same working conditions for all wells.
8. Calibrate the pipette frequently to assure the accuracy of specimens/reagents dispensing. Use different disposal pipette tips for each specimen and reagents in order to avoid cross-contaminations.
9. Assure that the incubation temperature is 37°C inside the incubator.
10. When adding specimens, do not touch the well's bottom with the pipette tip.
11. When measuring with a plate reader, determine the absorbance at 450nm or at 450/600~650nm.
12. The enzymatic activity of the HRP-conjugate might be affected from dust and reactive chemical and substances like sodium hypochlorite, acids, alkalis etc. Do not perform the assay in the presence of these substances.
13. If using fully automated equipment, during incubation, do not cover the plates with the plate cover. The tapping out of the remainders inside the plate after washing, can also be omitted.
14. All specimens from human origin should be considered as potentially infectious. Strict adherence to GLP (Good Laboratory Practice) regulations can ensure the personal safety.

15. Chemical should be handled and disposed of only in accordance with the current GLP (Good Laboratory Practices) and the local or national regulations.
16. The pipette tips, vials, strips and specimen containers should be collected and autoclaved for not less than 2 hours at 121°C or treated with 10% sodium hypochlorite for 30 minutes to decontaminate before any further steps of disposal. Solutions containing sodium hypochlorite should NEVER be autoclaved. Materials Safety Data Sheet (MSDS) available upon request.
17. Some reagents may cause toxicity, irritation, burns or have carcinogenic effect as raw materials. Contact with the skin and the mucosa should be avoided but not limited to the following reagents: Stop solution, the Chromogen, and the Wash buffer.
18. The Stop solution 2M H2SO4 is an acid. Use it with appropriate care. Wipe up spills immediately and wash with water if come into contact with the skin or eyes.
19. ProClin 300 0.5% used as preservative, can cause sensation of the skin. Wipe up spills immediately or wash with water if come into contact with the skin or eyes.

Limitations

1. Positive results must be confirmed with another available method and interpreted in conjunction with the patient clinical information.
2. Antibodies may be undetectable during the early stage of the disease and in some immunosuppressed individuals. Therefore, negative results obtained with SARS-CoV-2 Total Antibody ELISA are only indication that the specimen does not contain detectable level of antibodies and any negative result should not be considered as conclusive evidence that the individual is not infected with the virus.
3. If, after retesting of the initially reactive specimens, the assay results are negative, these specimens should be considered as non-repeatable (false positive) and interpreted as negative. As with many very sensitive ELISA assays, false positive results can occur due to the several reasons, most of which are related but not limited to inadequate washing step.
4. The most common assay mistakes are: using kits beyond the expiry date, bad washing procedures, contaminated reagents, incorrect assay procedure steps, insufficient aspiration during washing, failure to add specimens or reagents, improper operation with the laboratory equipment, timing errors, the use of highly hemolysis specimens or specimens containing fibrin, incompletely clotted serum specimens.
5. The prevalence of the marker will affect the assay's predictive values.
6. This assay cannot be utilized to test pooled (mixed) serum or plasma. The kit has been evaluated only with individual serum or plasma specimens.
7. SARS-CoV-2 Total Antibody ELISA is a qualitative assay and the results cannot be used to measure antibody concentration.