（FOR RESEARCH USE ONLY. DO NOT USE IT IN CLINICAL DIAGNOSTICS !）

Catalog No: E-EL-E607

Product size: 24T/96T/96T*5

SARS-CoV-2 Spike Protein Total Antibody ELISA Kit

This manual must be read attentively and completely before using this product.

If you have any problems, please contact our Technical Service Center for help (info in the header of each page).

GENTAUR

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Please kindly provide us with the lot number (on the outside of the box) of the kit for more efficient service.

Intended use

This ELISA kit applies to the in vitro qualitative determination of total antibodies against SARS-CoV-2 Spike Protein in serum or plasma.

Test principle

This ELISA kit uses the Sandwich-Ag ELISA principle. The micro ELISA plate provided in this kit has been pre-coated with recombinant SARS-CoV-2 Spike Protein. After adding samples (or controls) to wells, the total antibodies against SARS-CoV-2 Spike Protein will combine with the pre-coated SARS-CoV-2 Spike Protein. After washing completely, add Horseradish Peroxidase (HRP) conjugated SARS-CoV-2 Spike Protein to develop the antigen-antibody-HRP conjugated antigen complex. Free components are washed away. The substrate solution is added to each well. Only those wells that contain SARS-CoV-2 Spike Protein, antibodies against SARS-CoV-2 Spike Protein and HRP conjugated SARS-CoV-2 Spike Protein will appear blue in color. The enzyme-substrate reaction is terminated by the addition of stop solution and the color turns yellow. The optical density (OD) is measured spectrophotometrically at a wavelength of 450 ±2 nm. Compared with the CUT OFF value to judge whether SARS-CoV-2 Spike Protein total antibody exists in the tested samples or not.

Kit components & Storage

An unopened kit can be stored at 2-8℃ for 1 month. If the kit is not supposed to be used within 1 month, store the items separately according to the conditions on the next page once the kit is received.

Other supplies required

Microplate reader with 450 nm wavelength filter
High-precision transfer pipette, EP tubes and disposable pipette tips
Incubator capable of maintaining 37℃
Deionized or distilled water
Absorbent paper
Loading slot

Item	Specifications	Storage
Micro ELISA Plate (Dismountable)	96T: 8 wells ×12 strips 24T: 8 wells ×3 strips 96T*5: 5 plates, 96T	-20℃, 6 months
Positive Control	96T: 2 vials 24T: 1 vial 96T*5: 10 vials	
Negative Control	96T: 2 vials 24T: 1 vial 96T*5: 10 vials	
Concentrated HRP Conjugated Spike Protein (HRP-SP, 100×)	96T: 1 vial, 120 μL 24T: 1 vial, 60 μL 96T*5: 5 vials, 120 μL	-20℃(Protect from light), 6 months
Sample & Control Diluent	96T/24T: 1 vial, 20 mL 96T*5: 5 vials, 20 mL	2-8℃, 6 months
HRP Conjugate Diluent	96T/24T: 1 vial, 14 mL 96T*5: 5 vials, 14 mL	
Concentrated Wash Buffer(25×)	96T/24T: 1 vial, 30 mL 96T*5: 5 vials, 30 mL	
Substrate Reagent	96T/24T: 1 vial, 10 mL 96T*5: 5 vials, 10 mL	2-8℃(Protect from light)
Stop Solution	96T/24T: 1 vial, 10 mL 96T*5: 5 vials, 10 mL	2-8℃
Plate Sealer	96T/24T: 5 pieces 96T*5: 25 pieces	
Product Description	1 copy	
Certificate of Analysis	1 copy	

Note: All reagent bottle caps must be tightened to prevent evaporation and microbial pollution. The volume of reagents in partial shipments is a little more than the volume marked on the label, please use accurate measuring equipment instead of directly pouring into the vial(s).

Sample collection

Serum: Allow samples to clot for 1 hour at room temperature or overnight at 2-8℃before centrifugation for 20 min at 1000×g at 2-8℃. Collect the supernatant to carry out the assay.
Plasma: Collect plasma using EDTA-Na_2 as an anticoagulant. Centrifuge samples for 15 min at 1000×g at 2-8℃ within 30 min of collection. Collect the supernatant to carry out the assay.

Note

■ **Note for kit**

1) For research use only.
2) Please wear lab coats, eye protection and latex gloves for protection. Please perform the experiment following the national security protocols of biological laboratories, especially when detecting blood samples or other bodily fluids.
3) A freshly opened ELISA plate may appear a water-like substance, which is normal and will not have any impact on the experimental results. Return the unused wells to the foil pouch and store according to the conditions suggested in the above table.
4) The microplate reader should be able to be installed with a filter that can detect the wave length at 450 ±10 nm. The optical density should be within 0-3.5. Follow the Instructions of the Microplate Reader for set-up and preheat it for 15 min before OD measurement.
5) Do not mix or substitute reagents with those from other lots or sources.
6) Change pipette tips in between adding of each standard level, between sample adding and between reagent adding. Also, use separate reservoirs for each reagent.
7) The kit should not be used beyond the expiration date on the kit label.

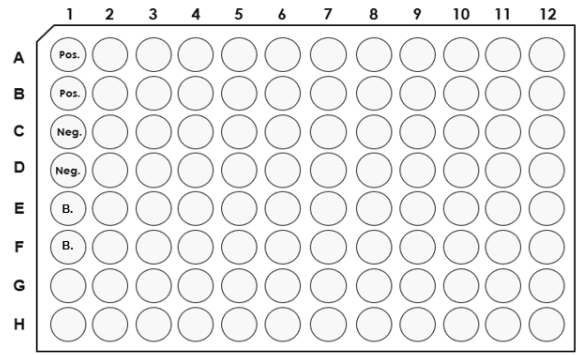
■ **Note for sample**

1) Tubes for blood collection should be disposable and be non-endotoxin. Severe hemolysis, lipoid, or turbidity samples should not be used.
2) Handle all serum and plasma as if capable of transmitting infectious agents.
3) Samples should be assayed within 3 days when stored at 2-8℃, otherwise samples must be divided up and stored at -20℃ (≤ 1 month) or -80℃ (≤ 3 months). Avoid repeated freeze-thaw cycles. Prior to assay, the frozen samples should be slowly thawed and centrifuged to remove precipitates. Frozen samples must be mixed well and brought to room temperature before testing.

Reagent preparation

1. Bring all reagents to room temperature (18-25℃) before use. If the kit will not be used up in one assay, please only take out the necessary strips and reagents for present experiment, and store the remaining strips and reagents at required condition.
2. **Wash Buffer:** Dilute 30 mL of Concentrated Wash Buffer with 720 mL of deionized or distilled water to prepare 750 mL of Wash Buffer. Note: if crystals have formed in the concentrate, warm it in a 40℃ water bath and mix it gently until the crystals have completely dissolved.
3. **HRP Conjugated Spike Protein working solution:** Calculate the required amount before the experiment (100 μL/well). In preparation, slightly more than calculated should be prepared. Centrifuge the Concentrated HRP Conjugate Spike Protein at 800×g for 1 min, then dilute the 100× **Concentrated HRP Conjugated Spike Protein** to 1× working solution with **HRP Conjugated Diluent** (Concentrated HRP-SP: HRP Conjugated Diluent= 1: 99). Note: The HRP-SP working solution should be stored at 2-8℃ and used within 1 day.
4. **Samples**: Dilute the tested serum or plasma at least 10 fold by using the **Sample & Control Diluent**, mix thoroughly.
5. **Positive Control/ Negative Control working solution**: Centrifuge the Controls at 10,000×g for 1 min. Add 0.5 mL of Sample & Control Diluent, let it stand for 10 min and invert it gently several times. The Control working solution should be stored at 2-8℃ and used within 1 day.

Pipetting protocol

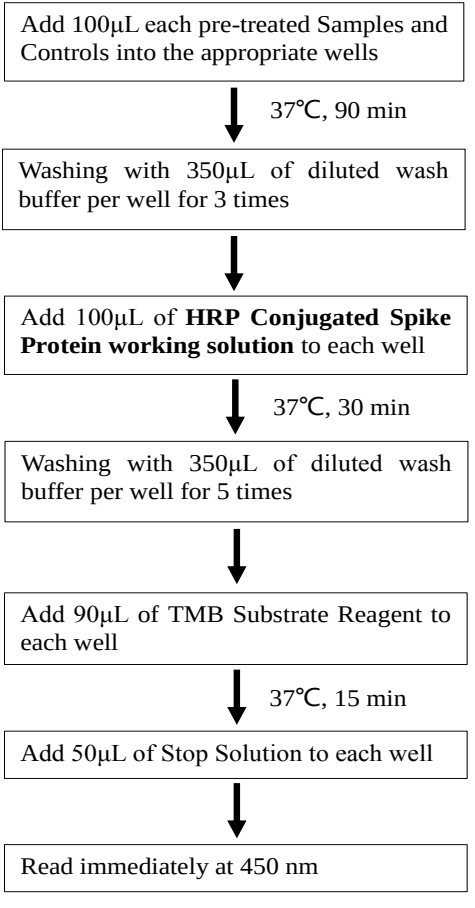


Pos.: Positive Control; **Neg.**: Negative Control; **B.**: Blank;

Assay procedure

1. Determine wells for **Positive Control**, **Negative Control, Blank** (Do not add any reagents except Substrate Reagent and Stop Solution) and **Sample**. Add 100μL of pre-treated Samples and Controls into the appropriate wells (It is recommended that all samples, Controls and blank be assayed in duplicate). Cover the plate with the sealer provided in the kit. Incubate for 90 min at 37℃. Note: solutions should be added to the bottom of the micro TEST plate well, avoid touching the inside wall and causing foaming as much as possible.
2. Decant the solution from each well, add 350μL of **wash buffer** to each well. Soak for 1-2 min and aspirate or decant the solution from each well and pat it dry against clean absorbent paper. Repeat this wash step 3 times. Note: a microplate washer can be used in this step and other wash steps. Make the tested strips in use immediately after the wash step. Do not allow wells to be dry.
3. Add 100μL of **HRP Conjugated Spike Protein working solution** to each well (except the blank well). Cover with the Plate sealer. Incubate for 30 min at 37℃.
4. Decant the solution from each well, repeat the wash process for 5 times as conducted in step 2.
5. Add 90μL of **Substrate Reagent** to each well. Cover the plate with a new sealer. Incubate for about 15 min at 37℃. Protect the plate from light. Note: the reaction time can be shortened or extended according to the actual color change, but not more than 30min. Preheat the Microplate Reader for about 15 min before OD measurement.
6. Add 50μL of **Stop Solution** to each well. Note: adding the stop solution should be done in the same order as the substrate solution.
7. Determine the optical density (OD value) of each well at once with a micro-plate reader set to 450 nm.

Assay Procedure Summary



Calculation

1. **Calculation method**
   (1) Use each assay result independently, determination of result according to Cut Off value.
   (2) Calculate the Cut Off: Cut Off = Negative Control average A_{450} × 2.1.
2. **Quality control**
   (1) Blank well (add substrate reagent and stop solution only) absorbance ≤ 0.08.
   (2) Positive Control A_{450} ＞1.0.
   (3) Negative Control A_{450} ≤ 0.18.
   Note: Experimental result is valid if any quality control is valid.
3. **Judgment method**
   (1) Positive result: Sample absorbance ≥ Cut Off
   The tested sample is classified as positive for total antibodies against SARS-CoV-2 Spike Protein.
   (2) Negative result: Sample absorbance ＜Cut Off
   The tested sample is classified as negative for total antibodies against SARS-CoV-2 Spike Protein.

Performance

Intra-CV: CV%＜8%

3 samples with low, mid-range and high level were tested 20 times on one plate respectively.

Inter-CV: CV%＜10%

3 samples with low, mid-range and high level were tested on 3 different plates, 20 replicates in each plate.

Declaration

1. Limited by current conditions and scientific technology, we can't conduct comprehensive identification and analysis on all the raw material provided. So there might be some qualitative and technical risks for users using the kit.
2. This assay is designed to eliminate interference by factors present in biological samples. Until all factors have been tested in the ELISA immunoassay, the possibility of interference cannot be excluded.
3. The final experimental results will be closely related to the validity of products, operational skills of the operators, the experimental environments and so on. We are only responsible for the kit itself, but not for the samples consumed during the assay. The users should calculate the possible amount of the samples used in the whole test. Please reserve sufficient samples in advance.
4. To get the best results, please only use the reagents supplied by the manufacturer and strictly comply with the instructions.
5. Incorrect results may occur because of incorrect operations during the reagents preparation and loading, as well as incorrect parameter settings of the Micro-plate reader. Please read the instructions carefully and adjust the instrument prior to the experiment.
6. Even the same operator might get different results in two separate experiments. In order to get reproducible results, the operation of every step in the assay should be controlled.
7. Every kit has strictly passed QC test. However, results from end users might be inconsistent with our data due to some variables such as transportation conditions, different lab equipment, and so on. Intra-assay variance among kits from different batches might arise from the above reasons, too.
8. Kits from different manufacturers or other methods for testing the same analyte could bring out inconsistent results, since we haven't compared our products with those from other manufacturers.
9. The kit is designed for research use only, we will not be responsible for any issues if the kit is applied in clinical diagnosis or any other related procedures.