

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

Catalog No: E-EL-E606

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

## **Elabscience® SARS-CoV-2 Neutralization 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).

Phone: 240-252-7368(USA) 240-252-7376(USA)

Email: [techsupport@elabscience.com](mailto:techsupport@elabscience.com)

Website: [www.elabscience.com](http://www.elabscience.com)

Please refer to specific expiry date from label outside of box.

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 Neutralization antibodies against SARS-CoV-2 in human serum or plasma.

## **Test principle**

This Test kit uses Competitive-ELISA as the method to qualitatively detect the Anti-SARS-CoV-2 Neutralization Antibody in the sample.

The micro ELISA plate provided in this kit is pre-coated with recombinant human ACE2. During the reaction, the SARS-CoV-2 Neutralization Antibody in the pretreated samples or controls competes with a fixed amount of human ACE2 on the solid phase supporter for sites on the Horseradish peroxidase (HRP) conjugated recombinant SARS-CoV-2 RBD fragment (HRP-RBD). After 37 °C incubation, the unbound HRP-RBD as well as any HRP-RBD bound to non-Neutralization antibody will be captured on the plate and eventually form the ACE2-RBD-HRP complex, while the circulating neutralization antibodies HRP-RBD complexes remain in the supernatant and are removed during washing. Then a TMB substrate solution is added to each well. The enzyme-substrate reaction is terminated by the addition of stop solution and the color change is measured spectrophotometrically at a wavelength of 450 nm  $\pm$  2 nm. Compared with the inhibition ratio to judge whether SARS-CoV-2 Neutralization Antibody exists in the tested samples or not.

## **Kit components & Storage**

The unopened kit can be stable for 6 months at 2-8 °C. After opening the kit, keep the reagents according to the conditions on the next page.

## **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 °C

Deionized or distilled water

Absorbent paper

Loading slot

Item	Specifications	Storage
Micro ELISA Plate (Dismountable)	24T: 8 wells $\times$ 3 strips 96T: 8 wells $\times$ 12 strips 96T*5: 5 plates, 96T	2-8°C, 1 week
Positive Control	24T: 1 vial 96T: 2 vials 96T*5: 10 vials	2-8°C, 6 months
Negative Control	24T: 1 vial 96T: 2 vials 96T*5: 10 vials	
Concentrated HRP Conjugated RBD (HRP-RBD, 100 $\times$ )	24T: 1 vial, 60 $\mu$ L 96T: 1 vial, 120 $\mu$ L 96T*5: 5 vials, 120 $\mu$ L	2-8°C(Protect from light), 6 months
Sample & Control Diluent	24T/96T: 1 vial, 20 mL 96T*5: 5 vials, 20 mL	2-8°C, 6 months
HRP Conjugated RBD Diluent	24T/96T: 1 vial, 14 mL 96T*5: 5 vials, 14 mL	
Concentrated Wash Buffer(25 $\times$ )	24T/96T: 1 vial, 30 mL 96T*5: 5 vials, 30 mL	
Substrate Reagent	24T/96T: 1 vial, 10 mL 96T*5: 5 vials, 10 mL	2-8°C(Protect from light)
Stop Solution	24T/96T: 1 vial, 10 mL 96T*5: 5 vials, 10 mL	2-8°C, 6 months
Plate Sealer	24T/96T: 3 pieces 96T*5: 15 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

(More detailed information please view our website: <https://www.elabscience.com/List-detail-259.html>)

**Serum:** Allow samples to clot for 1 hour at room temperature or overnight at 2-8°C before centrifugation for 20 min at 1000×g at 2-8°C. Collect the supernatant to carry out the assay.

**Plasma:** Collect plasma using EDTA-Na<sub>2</sub> as an anticoagulant. Centrifuge samples for 15 min at 1000×g at 2-8°C 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  $450 \pm 10$  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, lipid, 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°C, otherwise samples must be divided up and stored at -20°C ( $\leq 1$  month) or -80°C ( $\leq 3$  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°C) 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°C water bath and mix it gently until the crystals have completely dissolved.
3. **HRP-RBD working solution:** Calculate the required amount before the experiment (50 µL/well). In preparation, slightly more than calculated should be prepared. Centrifuge the Concentrated HRP Conjugate at 800×g for 1 min, then dilute the 100× Concentrated HRP-RBD to 1× working solution with **HRP Conjugated RBD Diluent**(Concentrated HRP-RBD: HRP Conjugated RBD Diluent= 1: 99). Note: The HRP-RBD working solution should be stored at 2-8°C and used within 1 day.
4. **Samples:** Dilute the tested serum or plasma at 10 fold by using the Sample & Control Diluent, mix thoroughly.
5. **Positive control:** Dissolve Positive Control with 0.3mL Sample & Control Diluent.
6. **Negative control:** Dissolve Negative Control with 0.5 mL Sample & Control Diluent.
7. Pre-treated Samples and Controls should be stored at 2-8°C and used within 1 day.

## Pipetting protocol

	1	2	3	4	5	6	7	8	9	10	11	12
A	Pos.											
B	Pos.											
C	Neg.											
D	Neg.											
E												
F												
G												
H												

**Pos.:** Positive Control;

**Neg.:** Negative Control;

## Assay procedure

1. Determine wells for **Positive** and **Negative Controls** and **samples**. Add 50 $\mu$ L each pre-treated Samples and Controls into the appropriate wells (It is recommended that all samples and Controls be assayed in duplicate). Immediately add 50 $\mu$ L of **HRP conjugated SARA-CoV-2 RBD fragment (HRP-RBD) working solution** to each well. Cover the plate with the sealer provided in the kit. Incubate for 60 min at 37 $^{\circ}$ C. 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 350 $\mu$ L of **wash buffer** to each well. Soak for 30-60 seconds 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 90 $\mu$ L of **Substrate Reagent** to each well. Cover the plate with a new sealer. Incubate for about 15 min at 37 $^{\circ}$ C. 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.
4. Add 50 $\mu$ L of **Stop Solution** to each well. Note: adding the stop solution should be done in the same order as the substrate solution.
5. Determine the optical density (OD value) of each well at once with a micro-plate reader set to 450 nm.

## Assay Procedure Summary

Add 50 $\mu$ L each pre-treated Samples and Controls into the appropriate wells



Immediately add 50 $\mu$ L of HRP-RBD working solution to each well



37°C, 60 min

Washing with 350  $\mu$ L of diluted wash buffer per well for 3 times



Add 90 $\mu$ L of TMB Substrate Reagent to each well



37°C, 15 min

Add 50 $\mu$ L of Stop Solution to each well



Read immediately at 450 nm

## Quality control

For each assay, both Positive and Negative Controls must be included to validate the results. The OD450 of each Control must meet the requirements as follows, otherwise, the test is invalid and should be repeated.

OD of Negative Control > 1.2

OD of Positive Control < 0.4

## Interpretation

The OD of the Negative Control is used to calculate the inhibition, and the OD of Positive Control is only used to evaluate the validity of the results. The inhibition of each sample can be calculated with the formulation as follows:

$$\text{Inhibition} = \left( 1 - \frac{\text{OD value of Sample}}{\text{OD value of Negative Control}} \right) \times 100\%$$

**Inhibition ≥ 20%:** Positive, Neutralization antibodies for SARS-CoV-2 are detected.

**Inhibition < 20%:** Negative, Neutralization antibodies for SARS-CoV-2 are not detected.

## Analytical performance

1. Repeatability: the CV ≤ 15%
2. Analysis specificity: There is no cross-reaction with antibody/antigen positive sera samples from patients with other human coronaviruses (HCoV-HKU1, HCoV- OC43, HCoV-NL63, HCoV-229E), or non-coronaviruses, including influenza A virus (H1N1, H3N2, H5N1, H7N9), influenza B virus ( Yamagata lineages, Victoria lineages), respiratory syncytial virus, rhinovirus, adenovirus, enterovirus, Epstein-Barr virus, measles virus, human cytomegalovirus, rotavirus, norovirus, mumps virus, herpes zoster virus, or Mycoplasma pneumoniae.
3. The cut-off value was determined at 98% sensitivity of 500 healthy people sera, as < 20%.



## **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.