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- Trockeneiszuschlag
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- Expressversand

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## **SARS-CoV-2 Nucleocapsid Protein IgG ELISA Kit**

Catalog No: E-EL-E600

96T

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.

Phone: 240-252-7368(USA) Fax: 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 on the side of box.

Please kindly provide us 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 SARS-CoV-2 Nucleocapsid Protein IgG in serum or plasma from pneumonia patients infected with SARS-CoV-2.

**Test principle**

This ELISA kit uses Indirect-ELISA as the method to qualitatively detect the SARS-CoV-2 Nucleocapsid protein IgG in the sample. The micro ELISA plate provided in this kit is pre-coated with purified SARS-CoV-2 Nucleocapsid protein antigen, after adding samples to wells, the SARS-CoV-2 Nucleocapsid protein IgG in the samples will combine with the pre-coated SARS-CoV-2 Nucleocapsid protein antigen. After washing completely, add Horseradish Peroxidase (HRP) conjugated mouse anti human IgG to develop the antigen-antibody-HRP conjugated secondary antibody complex. Free components are washed away, then the substrate solution is added to each well. Only those wells that contain SARS-CoV-2 Nucleocapsid protein IgG and HRP conjugated anti-human IgG 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  $450 \pm 2$  nm. Compared with the CUT OFF value to judge whether SARS-CoV-2 Nucleocapsid protein IgG exists in the tested samples or not.

**Kit components**

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

Item	Specifications	Storage
Micro ELISA Plate (Dismountable)	8 wells ×12 strips	2-8°C, 6 months
Positive Control	2 vials	2-8°C, 6 months
Negative Control	2 vials	
Sample & Control Diluent	1 vial, 25 mL	
Concentrated HRP Conjugated Mouse anti-human IgG (100×)	1 vial, 120 μL	2-8°C (Protect from light), 6 months
HRP Conjugate Diluent	1 vial, 14 mL	2-8°C, 6 months
Concentrated Wash Buffer (25×)	1 vial, 30 mL	
Stop Solution	1 vial, 10 mL	
Substrate Reagent	1 vial, 10 mL	2-8°C (Protect from light), 6 months
Plate Sealer	5 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).

### **Other supplies required**

Microplate Reader with 450 nm wavelength filter or dual-wavelength (450/630 nm)

High-precision transfer pipettor, EP tubes and disposable pipette tips

Incubator capable of maintaining 37°C

Deionized or distilled water

Absorbent paper

Loading slot for Wash Buffer

### **Note**

1. 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.
2. A freshly opened ELISA Plate may appear to have 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 provided in the kit, store it according to the conditions suggested in the above table.
3. 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.
4. Do not mix or use components with those from other lots.
5. Change pipette tips in between adding of each standard level, between sample adding and between reagent adding. Also, use separate reservoirs for each reagent.

## Sample collection and preparation

**Serum:** Allow samples to clot for 2 hours 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. The suspended fibrous protein may cause a false positive result if not fully precipitated. Obviously contaminated samples can't be detected.

**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 to carry out the assay.

**Before test, prepare the samples as the following instructions.**

**Sample dilution:** Dilute the tested serum or plasma at 100 fold by using the Sample & Control Diluent, mix thoroughly.

## Note for samples

1. Tubes for blood collection should be disposable and be non-endotoxin. Samples with high hemolysis or much lipid are not suitable for ELISA assay.
2. Samples should be assayed within 7 days when stored at 2-8 °C, otherwise samples must be divided up and stored at -20 °C (≤1 month) or -80 °C (≤3 months). Avoid repeated freeze-thaw cycles. Prior to assay, the frozen samples should be slowly thawed and centrifuged to remove precipitates.

## 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. **Positive Control and Negative Control working solution:** Centrifuge the Positive Control or Negative Control 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. After it dissolves fully, mix it thoroughly with a pipette.
3. **Concentrated HRP Conjugated Mouse anti-human IgG working solution:** Calculate the required amount before the experiment (100 μL/well). In preparation, slightly more than calculated should be prepared. Dilute the 100× **Concentrated HRP Conjugated Mouse anti-human IgG** to 1× working solution with HRP Conjugate Diluent.
4. **Wash Buffer:** Dilute 30mL of Concentrated Wash Buffer with 720mL of deionized or distilled water to prepare 750mL 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.

## Assay procedures

1. Determine wells for **Positive Control**, **Negative Control**, **Blank** (Do not add any reagents except Substrate Reagent and Stop Solution) and **Samples**. Add 100 μL of controls and samples to the appropriate wells (It is recommended that all controls and samples be assayed in duplicate.). Cover the plate with the sealer provided in the kit. Incubate for 45 min at 37 °C. Note: solutions should be added to the

- bottom of the micro ELISA 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 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 100 $\mu$ L of **HRP Conjugated Mouse anti-human IgG** working solution to each well (except the blank well). Cover with the Plate sealer. Incubate for 30 min at 37°C.
  4. Decant the solution from each well, repeat the wash process for 5 times as conducted in step 2.
  5. Add 90 $\mu$ L of **Substrate Reagent** to each well (including the blank well). Cover with a new plate sealer. Incubate for about 15 min at 37°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. Pre-heat the Microplate Reader for about 15 min before OD measurement.
  6. Add 50 $\mu$ L of **Stop Solution** to each well (including the blank 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.

## Calculation

### 1. Calculation method

- (1) Use each assay result independently, determination of result according to Cut Off value.
- (2) Calculate the Cut Off:  $\text{Cut Off(C.O.)} = 0.13 + \text{negative control(NC) average A450}$ . When  $\text{NC average A450} < 0.05$ , calculate it as 0.05; while  $0.05 \leq \text{NC average A450} \leq 0.10$ , calculate it as the actual value.

### 2. Quality control

- (1) Blank well (add substrate reagent and stop solution only) absorbance  $\leq 0.08$ .
- (2) Positive control (PC) A450  $> 0.60$ .
- (3) Negative control (NC) A450  $\leq 0.10$ .

**Experimental result is valid if any quality control is valid.**

### 3. Judgment method

- (1) Positive result: Sample absorbance  $\geq$  Cut Off  
The tested sample is classified as positive for SARS-CoV-2 Nucleocapsid protein IgG.
- (2) Negative result: Sample absorbance  $<$  Cut Off  
The tested sample is classified as negative for SARS-CoV-2 Nucleocapsid protein IgG.

## 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.