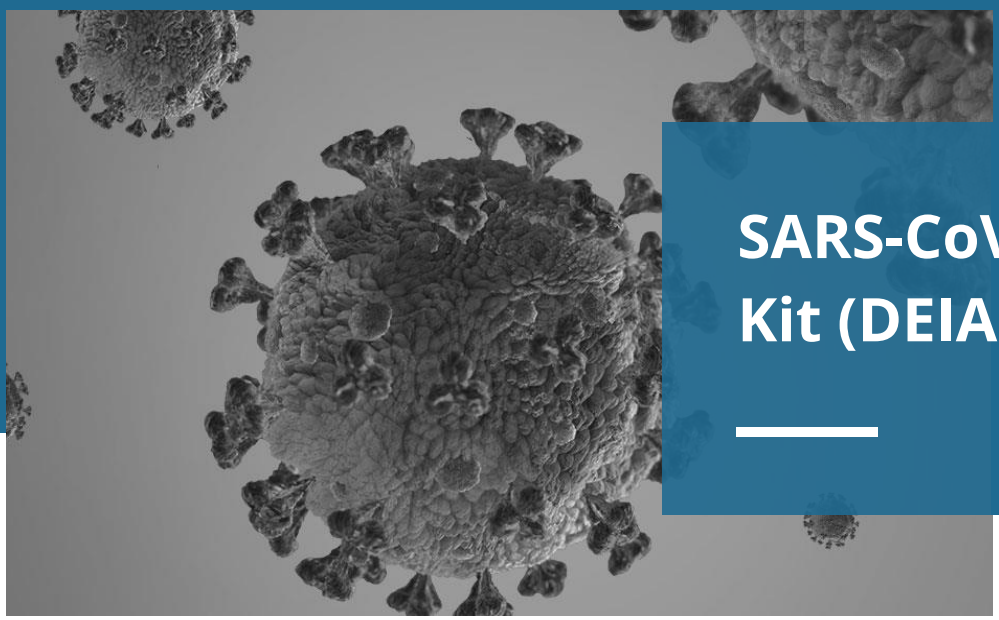

# SARS-CoV-2 IgG ELISA Kit (DEIASL019)

This product is for research use only and is not intended for diagnostic use.

## PRODUCT INFORMATION

**Size** 96T

### Intended Use

This kit is used for the qualitative detection of novel coronavirus IgG antibodies in human serum or plasma in vitro.

### General Description

The new coronavirus belongs to the beta coronavirus of the genus β, which has an envelope, the particles are round or oval, often polymorphic, and the diameter is 60-140nm. Its genetic characteristics are significantly different from SARSr-CoV and MERSr-CoV. Current research shows that it has more than 85% homology with bat SARS-like coronavirus (bat-SL- CoVZC45). In vitro isolation and culture, 2019-nCoV can be found in human respiratory epithelial cells in about 96 hours, while it takes about 6 days to isolate and culture in Vero E6 and Huh-7 cell lines. Based on current epidemiological investigations, the incubation period is generally 7 days, with a maximum of 14 days. Main symptoms are fever, fatigue, and dry cough. A few patients have symptoms such as nasal congestion, runny nose, and diarrhea. In severe cases, dyspnea occurs more than a week later. In severe cases, acute respiratory distress syndrome, septic shock, difficult to correct metabolic acidosis, and coagulation dysfunction develop rapidly. It is worth noting that in the course of severe and critically ill patients, there may be moderate to low fever, even without obvious fever. Some patients showed only low fever, mild fatigue, and no pneumonia and recovered after 1 week. In the early stages of the disease, the total number of white blood cells in the peripheral blood was normal or decreased, the lymphocyte count decreased, and some patients had increased liver enzymes, muscle enzymes, and myoglobin. Most patients have elevated C- reactive protein (CRP) and erythrocyte sedimentation rate and normal procalcitonin. In severe cases, D-dimer increases and peripheral blood lymphocytes progressively decrease. New

coronavirus nucleic acids can be detected in throat swabs, sputum, lower respiratory tract secretions, and blood. [1] Serum antibody testing helps confirm the infection status of a case.

## Principles of Testing

This kit is based on the principle of indirect method (ELISA) to detect the SARS-COV-2 IgG antibody in human serum or plasma. The SARS-COV-2 whole virus lysate antigen is pre-coated on the enzyme-labeled strips. Add the test sample and incubate it. The IgG antibody in the sample is bound to the antigen. Wash the plate to remove SARS-COV-2 IgG that does not bind to the coated antigen. Add the enzyme-labeled reagent for a second incubation. When a SARS-COV-2 IgG antibody is present in the sample, a "coated antigen-IgG antibody-anti-human IgG enzyme conjugate" complex will be formed. After washing the plate again, color reagent is added, and the HRP linked to the complex will catalyze the color The reagent reacts to produce a blue product, which turns yellow after the reaction is terminated; if no SARS-COV-2 IgG antibody is present in the sample, it does not develop a color. Measure the OD value on a microplate reader or enzyme immunoassay system, and determine the presence or absence of a SARS-COV-2 IgG antibody based on the OD value.

## Reagents And Materials Provided

1. Microplate: Purified virus lysate coating plate, 96 well. Ready to use.
2. Negative Control: 0.5 mL. Human serum with stabilizers and preservatives.
3. Positive Control: 0.2 mL, Human serum with stabilizers and preservatives.
4. Enzyme Solution: 13 mL, Horseradish-labeled anti-human IgG antibody with preservatives.
5. Wash Concentrate(20x): 30 mL. PBST with the right amount of preservatives.
6. Sample Diluent: 13 mL.
7. Substrate A: 8 mL.
8. Substrate B: 8 mL.
9. Stop Solution: 8 mL.
10. Plate Cover: 3 pcs

## Materials Required But Not Supplied

1. Precision single channel pipettes capable of delivering 20 µL, 25 µL, 100 µL, and 1000 µL, etc.
2. ELISA multichannel wash bottle or automatic (semi-automatic) washing system.
3. Spectrophotometric microplate reader capable of reading absorbance at 450 nm.
4. Distilled water.
5. Timer.
6. 37 °C incubator.
7. Laboratory safety equipment, such as disposable gloves.

## Storage

This kit is stored at 2 ~ 8 °C, the validity period is 6 months.

Avoid freezing and use within the validity period.

Production date, valid until the label.

## Specimen Collection And Preparation

* Sample type: human serum, plasma.
* Sample collection: The collection and testing of patient blood samples must be performed in accordance with the "Technical Guidelines for Laboratory Testing of New Coronavirus Infected Pneumonia" (third edition) issued by the National Health and Health Commission.
* Sample storage: After the blood sample is collected, the sample should be separated and tested in time; if the sample cannot be detected in time, the sample should be stored in accordance with the "Technical Guidelines for Laboratory Testing of Pneumonia of New Coronavirus Infection" (third edition) issued by the National Health and Health Commission.
* Sample safety: All samples are regarded as potentially infectious items and strictly implemented in accordance with relevant national standards and guidelines.

## Reagent Preparation

20X concentrated washing solution: Take the required amount from the bottle with a clean pipette and dilute it with purified water 1:19 to become a washing solution for later use. Example: Take 1mL of concentrated washing solution and dilute with 19mL of purified water. After dilution, the buffer solution is stable at 2 ~ 8 °C for up to one week. If crystals appear in the 20-fold concentrated washing solution, it should be heated to 37 °C and fully dissolved and mixed before dilution.

## Assay Procedure

1. Sample incubation

   Set blank, positive control 1 well, and negative control 2 wells in each experiment. No liquid is added to the blank wells. The 100 µL of negative and positive control is directly added to the positive and negative control wells without dilution. 100 µL of sample dilution is added to the remaining test wells. Add another 10µL of the sample to be tested, mix thoroughly, sealed with the cover film, and incubate at 37 °C for 30 minutes.

2. Wash plate

   Manual washing operation: add 300 µL of washing solution to each well, leave it to stand for 5-10 seconds, discard it, and rinse it 5 times, then pat dry;

   Washing machine operation: add 300-350 µL of washing solution to each well, and the washing interval is 5-10 seconds. After repeated washing 5 times, pat dry.

3. Incubate with enzyme working solution

   Add 100 µL of enzyme working solution to each well, sealed with the cover film, and incubate at 37 °C for 20 minutes.

4. Wash plate

   Manual washing operation: add 300 µL of washing solution to each well, leave it to stand for 5-10 seconds, discard it, and rinse it 5 times, then pat dry;

   Washing machine operation: add 300-350 µL of washing solution to each well, and the washing interval is 5-10 seconds. After repeated washing 5 times, pat dry.

5. Color reaction

   Add 50 μL of Substrate A and 50 μL of Substrate B to each well, pat gently, mix and place at 37 °C in the dark for 10 minutes.

6. Stop reaction

   After the color development is completed, add 50 μL of stop solution to each well and pat gently to mix.

7. Reading results

   Immediately after stopping the reaction, measure the OD value at 450nm wavelength (zeroed with blank holes) or dual- wavelength 450nm / 620nm on the microplate reader.

## Quality Control

Each test should meet the OD value of the positive control ≥ 0.50 and the OD value of the negative control ≤ 0.10 at the same time; otherwise, the test results are considered invalid.

## Interpretation of Results

Cut-off (cut off value) calculation: cut off value = 0.10 + negative control

(Mean of negative control OD, if less than 0.05, calculate as 0.05)

**Positive:** If the OD value of the tested sample is greater than the cut-off value, it should be judged as positive for new-type coronavirus (SARS-COV-2) IgG antibody.

**Negative:** If the OD value of the tested sample is less than the cutoff value, it is judged as negative.

It is recommended to re-test near the cut-off value. If the re-test result is positive, it is judged as positive, otherwise it is judged as negative. Weakly positive samples for this product should be tested by other methods to exclude false positives.

**Notes:**

1. Insufficient washing is the main cause of false positives;
2. Other operational errors may lead to false positives and false negatives of the test results, such as: the kit is used outside the validity period, the sampler is inaccurate, the indoor temperature is too low, and the test is not performed according to the testing procedures of the instructions
3. The determination of positive results and negative results should be determined jointly based on clinical characteristics and other detection indicators.

## Limit of Detection

3 reference products with minimum detection limits were tested, all of which were positive.

## Reproducibility

Inter-assay: The assay control is tested in 10 replicates with a CV of OD values less than 15%.

Intra-assay: Three lots were tested with the same samples 10 times with a CV less than 20%.

## Sensitivity & Specificity

Serum samples from two cohorts of patients were tested using the IgG ELISA Kit. The combined cohort consisted of normal healthy patients with samples collected prior to the SARS-COV-2 outbreak (n=30) and RT- PCR confirmed positive patients in after the second week of the onset of the disease (n=16). The results are as follows:

| | Test Positive | Test Negative |
|---|---|---|
| Confirmed Positive | 16 | 0 |
| Confirmed Negative | 0 | 30 |

The diagnostic sensitivity is 100%.

The diagnostic specificity is 100%.

## Limitations

1. According to the basic theory of antibody production after the body is infected, after the body is infected with a virus, specific IgM antibodies are produced earlier and have a shorter duration; IgG antibodies are produced later and have a longer duration than IgM antibodies. In addition, it takes a certain period of time from the infection of the virus to the production of specific antibodies by the body, and there are individual differences in the strength of the antibodies, which are related to the amount of the infected antigen and the antigenic strength of the antigen. Therefore, the IgG antibody test result, IgM antibody test result sampling time, clinical indications and appearance time should be considered together. Those who test positive for antibodies should also make a comprehensive judgment in conjunction with other clinical indications.
2. This strain uses a novel coronavirus (SARS-COV-2) isolated from specimens of "new coronavirus-infected pneumonia" cases that became prevalent in January 2020. The purified whole virus lysate is used as an antigen for ELISA Kit, but whether the virus strain used in this product is completely consistent with the current domestic epidemic strain requires further confirmation.
3. This test is a qualitative analysis test. The test results do not accurately reflect the titer of the new coronavirus (SARS- COV-2) IgG antibody.
4. This kit can only be used for the determination of serum or plasma samples, not other body fluid samples.

## Precautions

1. The negative and positive controls of this kit are derived from humans. Although they have been inactivated and tested for HBsAg, TP antibody, HCV antibody and HIV antibody by ELISA, they are negative, but they cannot be guaranteed to be free of potential viruses and other microorganisms. Infectious; there is no known test method that can fully prove that human blood samples will not cause infection. Therefore, the negative control and detection samples should be operated strictly in accordance with relevant biosafety regulations and the routine regulations of laboratory operations;
2. When all reagents are taken out from the refrigerated environment, they should be equilibrated to room temperature (10-30 °C) before use; the reagents should be shaken before use;
3. A micro-sampler should be used for each sample addition;
4. Each well should be filled with washing liquid during washing;

5. Sealant should be used once;
6. Do not mix reagents of different batches;
7. All samples and reagents should avoid direct contact with skin and eyes, and do not swallow; if this happens, immediately rinse with plenty of water and go to the hospital for treatment;
8. All samples, washing liquids and various wastes should be treated as pollutants.