Creative Diagnostics®

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**User's Manual**

# SARS-CoV-2 IgG Quantitative ELISA Kit

REF **DEIASL019Q**

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

For illustrative purposes only. To perform the assay the instructions for use provided with the kit have to be used.


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# PRODUCT INFORMATION

## Intended Use

This kit is used for the quantitative 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 60-140 nm. 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 nothing 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. 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 substrate. 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 concentration 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. Standard (lyophilized): 1 µg, SARS-CoV-2 IgG antibody.
3. Standard Diluent: 13 mL. Ready to use.
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.

## 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 1X washing solution for later use. Example: Take 1 mL of concentrated washing solution and dilute with 19 mL 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.

Standard Preparation: Dilute 1 μg lyophilized standard with 1 mL standard diluent to 1 μg/mL. The dissolved standard needs to be stored at -80°C and avoid repeated freeze-thaw cycles. Dilute 1 μg/mL spiking standard solution with standard diluent at the ratio of 1:4 to obtain 200 ng/mL standard solution (Standard A). Then dilute according to the table below to obtain standard solutions A-F.

| Standard | IgG Concentration (ng/mL) | Dilutions |
|---|---|---|
| A | 200 | 800μL Standard Diluent + 200μL 1μg/mL Spiking standard |
| B | 133.333 | 200μL Standard Diluent + 400μL Standard A |
| C | 88.889 | 200μL Standard Diluent + 400μL Standard B |
| D | 59.239 | 200μL Standard Diluent + 400μL Standard C |
| E | 39.506 | 200μL Standard Diluent + 400μL Standard D |
| F | 0 | 400μL Standard Diluent |

## Assay Procedure

1. Sample incubation

Add 100 μL of each serially diluted SARS-CoV-2 IgG antibody standards to the set wells. Add 100 μL of sample diluent to the remaining test wells. Add another 10 μL 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 1X 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 1X 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 1X 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 1X 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.

## Calculation

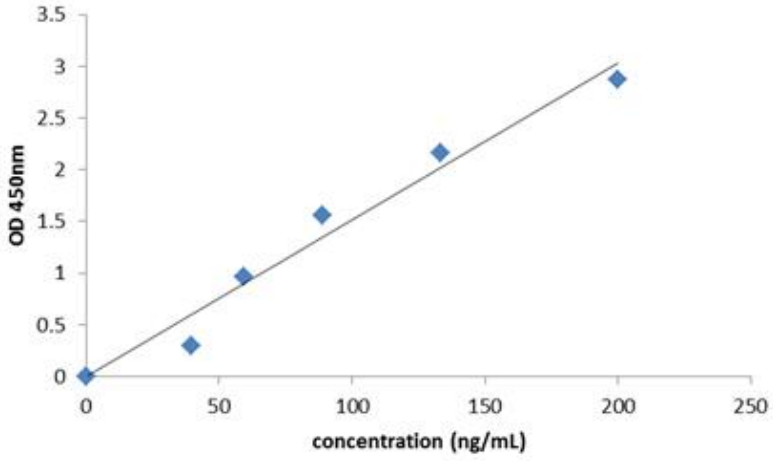
If samples generate values higher than the highest standard, dilute the samples and repeat the assay. Calculate the mean absorbance for each standard and sample and subtract average zero standard optical density (O.D.).

Construct a standard curve by plotting the mean absorbance for each standard on the y-axis against the concentration on the x-axis and draw a best fit curve through the points on the graph. Most graphing software can help make the curve and a four-parameter logistic (4-PL) usually provide the best fit, though other equations (e.g. linear, log/log) can also be tried to see which provides the most accurate. Extrapolate the target protein concentrations for unknown samples from the standard curve plotted.

## Typical Standard Curve

This standard curve is only for demonstration purposes. A standard curve should be generated for each assay.

| Standard | Concentration (ng/mL) | OD Value |
|---|---|---|
| A | 200 | 2.8719 |
| B | 133.333 | 2.1646 |
| C | 88.889 | 1.5559 |
| D | 59.259 | 0.9685 |
| E | 39.506 | 0.3012 |
| F | 0 | 0.0007 |




## Detection Range

The detection range of this kit is 39.506-200 ng/mL.

## Precautions

1. The kit should be taken out from the refrigerated environment and should be equilibrated to room temperature before being opened for use. The reagent should be thoroughly shaken before use.
2. The strips of the kit can be removed, and the unused pre-coated strips should be sealed in a desiccant bag.
3. Do not mix reagent components from different batches of the kit.
4. If crystals appear in the 20-fold concentrated washing solution, they should be placed at 37 ° C until the crystals are completely dissolved before use.
5. Read the results of the microplate reader within 30 minutes after the reaction is terminated.
6. This kit and all waste in the test are potentially contaminating and should be treated strictly in accordance with medical contamination.

## Limitations

1. This product is only used for testing of serum or plasma samples.
2. The test results of this product are for clinical reference only, and they should not be used as the sole basis for the diagnosis of new coronavirus.
3. Due to the window effect of the virus infection and the sensitivity of the kit detection, Samples collected from patients within 10 days after the onset of clinical symptoms should be evaluated with this assay (day 10 – day 15).