RBD Antibody (IgG) Quantitative and Qualitative Detection Kit (ELISA)

DD3112PEN

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Instruction for Use

Version 22.1

Vazyme

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Contents

01/Product Name

RBD Antibody (IgG) Quantitative and Qualitative Detection Kit (ELISA)

02/Specification

96 Tests

03/Intended Use

This kit is intended for the ELISA-based quantitative and qualitative detection of the anti-SARS-CoV-2 RBD IgG antibody in human serum or plasma samples.

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is the key pathogen of coronavirus disease 2019 (COVID-19). The virus is an enveloped, non-segmented, positive-sense single-stranded RNA virus of the Coronavirus genus in the Coronaviridae family. SARS-CoV-2 encodes a total of four major structural proteins, including the spike (S) protein, envelope (P) protein, membrane (M) protein, and nucleocapsid (N) protein. The S protein contains a receptor-binding domain (RBD), and it can bind to the host-cell surface receptor angiotensin-converting enzyme 2 (ACE2) to invade the host cell. SARS-CoV-2 infection is usually manifested by fever and different extents of respiratory symptoms such as cough, shortness of breath, and dyspnea. The incubation period is generally 2 - 14 days. Usually, the anti-SARS-CoV-2 IgM and IgG antibodies successively appear in the serum 2 - 3 weeks after the SARS-CoV-2 infection; thereafter, the IgM antibody level declines rapidly while the IgG antibody level keeps unchanged for a long time. This kit is only used for scientific research such as vaccine titer evaluation.

04/Test Principle

This kit is used for indirect ELISA-based detection of the anti-SARS-CoV-2 RBD IgG antibody during the two-step incubation. The microplate wells are pre-coated with the recombinant RBD protein that has the same amino acids as the target antigen. If the anti-SARS-CoV-2 RBD IgG antibody is present in the sample, it will bind to the coating antigen. Wash the plate to remove the unbound substance, and add the enzyme-labeled secondary antibody that can specifically bind to the human IgG antibody to form the "coating antigen-antibody-enzyme-labeled secondary antibody" complexes. Wash the plate again, and add the tetramethylbenzidine (TMB) substrate solution for color development. In the end, terminate the reaction and read the absorbance at the wavelength of 450 nm. The absorbance of sample is positively correlated with the anti-SARS-CoV-2 RBD IgG antibody level.

05/Key Components

Component	Cap Color	DD3112PEN-01
1. Pre-coated microplate (coated with the recombinant RBD protein)	-	12 × 8, 96 wells
2. Sample diluent	-	30 ml × 2
3. Standard 1: 0.0625 RU/ml	Yellow	1 ml
4. Standard 2: 0.125 RU/ml	Orange	1 ml
5. Standard 3: 0.25 RU/ml	Green	1 ml
6. Standard 4: 0.5 RU/ml	Blue	1 ml
7. Standard 5: 1 RU/ml	Purple	1 ml
8. Standard 6: 1.5 RU/ml	Red	1 ml
9. Standard 7: 2 RU/ml	Brown	1 ml
10. Concentrated wash buffer (80×)	-	30 ml
11. Enzyme-labeled reagent	-	12 ml
12. TMB substrate solution	-	12 ml
13. Stop solution	-	6 ml
14. Sealing film	-	3 pcs
15. Instructions for Use	-	1 pcs

Note: The components in this kit shall not be used interchangeably with those in other commercially available kits.

Reagents and consumables required but not provided:

> Deionized or distilled water
> Shaker
> Plate washer
> Micropipettes and compatible sterile tips
> Thermostatic incubator or water bath
> Microplate reader
> Sample loading slot
>Absorbent pad

06/Storage and Shelf Life

1. Store the kit at 2 ~ 8℃ and protect it from direct bright light. The kit has a shelf life of 12 months.
2. After a required number of pre-coated microplate strips are taken out, the remaining ones should be kept in the bag and stored at 2 ~ 8℃. The plate strips should be used within the shelf life.
3. The remainder of the other components in the kit should be put back at 2 ~ 8℃ quickly after use and should be used within the shelf life.
4. This kit should be refrigerated during transport.
5. For product lot number and expiration date, see the label on the outer packaging.

07/Sample Requirements

1. This kit is intended for use with serum/plasma samples.
2. The sediment and suspended matter in the sample may affect the test results and should be removed by centrifugation at 6,000 g for 10 minutes.
3. Grossly hemolyzed, lipemic, or turbid samples should be discarded.
4. Samples must be brought to room temperature (18 ~ 28℃) before testing. Frozen samples must be completely thawed, brought to room temperature, and thoroughly mixed before use. Repeated freeze-thaw cycles should be avoided.

08/Test Method

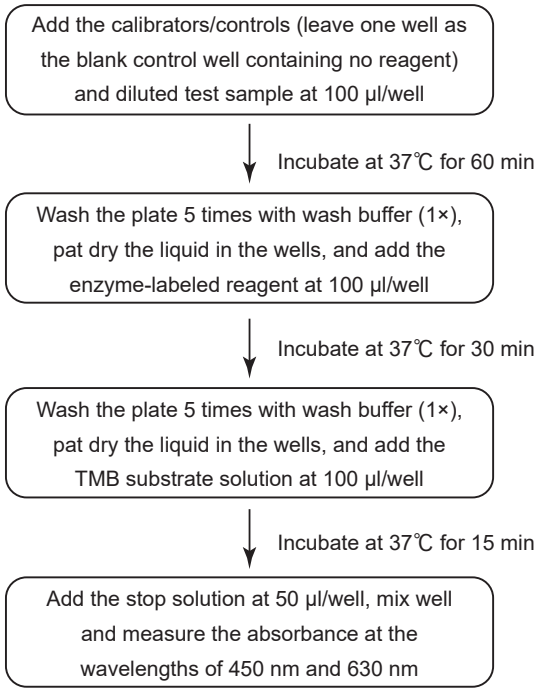
08-1/Test Preparation

1. Before the test, bring all reagents and samples to room temperature (for at least 30 minutes).
2. Adjust the temperature of the thermostatic incubator or water bath to be within the required temperature range.
3. Preparation of wash buffer (1×): Dilute the concentrated wash buffer (80×) with deionized or distilled water 80-fold, and mix well for later use. For example, dilute 30 ml of concentrated wash buffer (80×) with 2,370 ml of deionized or distilled water.
4. Preparation of control/standard
   4.1 Qualitative detection
   Use standard 7 as the positive control (PC) and the sample diluent as the negative control (NC), and add them at 100 µl/well.
   4.2 Quantitative detection
   Use standards 1 - 7 (100 µl/well) to establish the calibration curve.
5. Preparation of test sample
   5.1 Dilute the test sample with the sample diluent 10-fold (for example, dilute 10 µl of the sample with 90 µl of the sample diluent) and add it at 100 µl/well. If the dilution is not done in the pre-coated microplate, determine the appropriate dilution volume based on the loading volume to avoid the sample loss caused by dilution or transfer, which affects the final volume of the test sample participating in the reaction.
   5.2 If the test sample needs to be further analyzed, conduct a pre-test to determine the appropriate dilution or serial dilutions.

08-2/Test Procedures

1. Sample loading: Set one well as the blank control well (with no reagent added), and add the standards (for quantitative detection)/negative and positive controls (for qualitative detection) and the diluted test sample to the corresponding wells at 100 μl/well. It is recommended to design replicate wells for standards/negative and positive controls and the diluted test sample.
2. Incubation: Seal the plate with a sealing film, and incubate it in a 37℃ thermostatic incubator for 60 minutes.
3. Washing: Upon the completion of incubation, carefully remove the sealing film, and discard the liquid in the wells. Add at least 300 μl of wash buffer (1×) into each well, and leave it to soak for 30 seconds. Wash the plate 5 times, and remove all of the residual liquid to the extent possible in the last washing.
4. Sample loading: Add the enzyme-labeled reagent at 100 μl/well.
5. Incubation: Seal the plate with a sealing film, and incubate it in a 37℃ thermostatic incubator for 30 minutes.
6. Repeat Step 3.
7. Color development: Add the TMB substrate solution at 100 μl/well, seal the plate with a sealing film, and incubate it in a 37℃ thermostatic incubator away from light for 15 minutes.
8. Termination/Reading: Carefully remove the sealing film, add the stop solution at 50 μl/well, gently mix well, and load the mixture for reading the optical density (OD) value. If the wavelength can be calibrated, read the OD values at the wavelengths of 630 nm and 450 nm, and deduct the OD reading at 630 nm from that at 450 nm (correction of optical defects in the plate). If only the OD value at the single wavelength of 450 nm is measured, design a blank control well, and deduct the OD reading of the blank control well from the OD reading at 450 nm because the uncalibrated OD reading at 450 nm may be inaccurate.

09/Brief Operating Procedure



10/Quality Control

1. For the qualitative detection, the mean absorbance (A) value of negative control wells should be ≤ 0.10, while that of positive control wells should be ≥ 0.80. If there is only one negative control well with an A value greater than 0.10, discard this well; if there are two or more negative control wells with an A value greater than 0.10, repeat the test. Otherwise, the test is considered invalid.
2. For the quantitative detection, the correlation coefficient R^2 of the calibration curve should be ≥ 0.99; otherwise, the test is considered invalid.

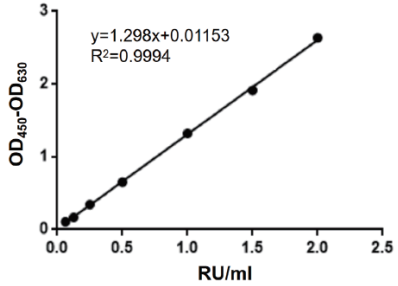
11/Result Calculation

11-1/Qualitative Detection

Calculation of the cut-off value: Cut-off value = 0.17 + Mean A value of negative control wells (If the mean A value is lower than 0.05, take it as 0.05).
(Note: A value = OD value at 450 nm - OD value at 630 nm or A value = OD value at 450 nm - OD value of the blank control well)

11-2/Quantitative Detection

1. Calculate the mean of A values (calculated as the OD difference between the two wavelengths) of replicate sample wells. Take the standard concentrations (RU/ml) as the x-coordinates and the A values as the y-coordinates to plot the calibration curve of the antibody standards using the linear regression equation. Use the calibration curve to calculate the antibody concentration of each test sample in the plate.
2. Limit of quantitation (LOQ) = 0.125 RU/ml. If the antibody concentration of a test sample is lower than 0.125 RU/ml, it should be reported to be < 0.125 RU/ml; if it is higher than the upper limit of detection range, it should be reported to be > 2 RU/ml. The calibration curve below is for demonstration purposes only, and a new calibration curve should be generated for each test.



12/Result Determination

Qualitative detection

1. An A value ≥ cut-off value indicates that the anti-SARS-CoV-2 RBD antibody (IgG) is detected in the test sample.
2. An A value < cut-off value indicates that the anti-SARS-CoV-2 RBD antibody (IgG) is not detected in the test sample.
3. For the test sample subject to gradient dilution, the highest dilution factor of the diluted test sample with the A value ≥ cut-off value is determined to be the antibody titer.

13/Result Interpretation

1. The standards provided in the kit are well correlated with the series diluted first WHO International Standard (IS) for anti-SARS-CoV-2 immunoglobulin (NIBSC code: 20/136, hereinafter referred to as the "WHO standard"), with r ≥ 0.99. 1 BAU/ml (WHO standard approximate value) = 1 RU/ml (Vazyme standard value).
2. When the original concentration of a diluted test sample is calculated based on the dilution factor, the measured A value of this sample should be no less than the cut-off value or LOQ. The antibody level of more than 2 RU/ml in a test sample is calculated by extending the calibration curve. For more accurate results, dilute this sample and test it again.

14/Limitations of Test Method

1. A negative test result or a test result that is lower than the LOQ cannot rule out the presence of RBD antibody, which is associated with the stage of infection or immunity or the sample dilution factor. Thus, this test must be combined with other assays and cannot be used as the only basis for determination.
2. A false-positive result may result from an earlier virus infection that produces antibodies with cross-reactivity with the target antigen.
3. The components in this kit may cross-react with the anti-SARS-CoV IgG antibody.
4. Grossly hemolyzed, lipemic, and icteric blood samples may cause serious errors in test results.
5. Strong acids, strong alkalis, strong oxidation, and strong reducing substances can change enzyme activity, so these extreme environmental conditions should be avoided throughout the test.

15/Performance Indicators

Precision: The coefficient of variation (CV) should be ≤ 15%.

16/Notes

For research use only. Not for use in diagnostic procedures.

1. Read the Instructions for Use carefully before operation and carry out the tests in strict accordance with it.
2. Avoid performing a test in harsh environments (e.g., environments containing dust and high-concentration corrosive substances such as chlorine-based disinfectant, sodium hypochlorite, acids, alkalis, and acetaldehyde). Disinfect the laboratory after the test.
3. Equilibrate the kit to room temperature before unpacking it, and shake the reagent well before use. Store and use each component in strict accordance with the Instructions for Use, and do not change or dilute the component arbitrarily. Carefully check the expiration date and packaging of the kit before use. If the kit expires or its package is damaged, do not use it for tests. Use the reagents within their shelf life, and seal and store the remainder according to the Instructions for Use.

4. The pre-coated microplate is removable. After taking off the required number of plate strips each time, keep the remainder in a ziplock bag and store it at 2 ~ 8°C for later use. Do not touch the bottom of the well when detaching the required strips from the plate to avoid fingerprints or scratches that may affect subsequent readings. After plate washing, immediately perform the next operation; otherwise, the plate may get dry and inactivated.
5. When loading the sample, avoid bubbles, and prevent the pipette tip from touching the bottom of the plate, which may cause scratches and affect the readings.
6. Do not re-use the sealing film. Do not use kit components with different lot numbers and micropipette tips interchangeably to avoid cross-contamination.
7. If crystals appear in the concentrated wash buffer, place the buffer at 37°C until crystals are dissolved before use. Fill up each well with wash buffer during washing to ensure the residual reagent in each well is removed. Wash the plate thoroughly. Do not apply too much force when loading the wash buffer to avoid solution contamination. Shake off the liquid in the wells after each plate washing (a plate washer is recommended), and pat it dry.
8. Read the result within 15 minutes after the reaction terminates.
9. Wear disposable gloves and protective gear in accordance with laboratory regulations during the operation. After the test, dispose of the liquid waste and disposable consumables in a harmless way in accordance with relevant local and national regulations.