Mouse Spike (Omicron) Antibody (IgG) Detection Kit (ELISA)

DD3208EN

Nanjing Vazyme Biotech Co.,Ltd.
Tel: +86 25-83772625
Email: info.biotech@vazyme.com
Web: www.vazyme.com
Loc: Red Maple Hi-tech Industry Park, Nanjing, PRC
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Instruction for Use

Version 23.1

Vazyme

Contents

01/Application

This kit is intended for ELISA-based titer determination of the spike (Omicron) IgG antibody in mouse serum.

02/Test Principle

This kit, based on the ELISA method, contains a pre-coated polystyrene microplate. The microplate wells are pre-coated with the recombinantly expressed and purified spike (Omicron) protein that is homologous to the amino acids of the target antigen. Incubate the mouse serum diluted with the sample diluent in the wells. In the presence of an antibody specific to the target protein, the specific antibody binds to the antigen in the solid phase. Wash the wells to remove non-specific binding complexes. Add the horseradish peroxidase (HRP)-labeled rabbit anti-mouse IgG antibody to wells. After the labeled detection antibody binds to the antigen-antibody complex in the wells, wash the wells to remove the labeled detection antibody left unbound. Add the TMB substrate solution to the wells. The development of blue color means the presence of specific antibodies. In the end, add the stop solution to terminate the reaction. Measure the absorbance (optical density, OD value) at 450 nm and 630 nm.

03/Key Components

No.	Component	DD3208EN-01
1	Pre-coated microplate (coated with the spike (Omicron) protein)	12 × 8，96 wells
2	Enzyme-labeled detection antibody (100×)	120 μl
3	Enzyme-labeled detection antibody diluent	12 ml
4	Sample diluent	2 × 12 ml
5	Concentrated wash buffer (20×)	30 ml
6	Positive control	0.5 ml
7	Negative control	0.5 ml
8	TMB substrate solution	12 ml
9	Stop solution	6 ml

Devices and reagents required but not provided in the kit:

- Microplate reader
- Deionized water
- Horizontal shaker
- Plate washer (optional) or manual washing
- Pipettes
- Pipette tips

- Reagent bottles (500 ml or 1 L)
- Thermostatic incubator
- Timer
- Absorbent pad
- Disinfectant for laboratory use

04/Storage and Shelf Life

1. Store the kit at 2 ~ 8°C, avoid freezing and protect it from bright light. The kit has a shelf life of 12 months.
2. The unpacked control can be stored at 2 ~ 8°C for 7 days. For longer-term storage, it should be aliquoted as needed for storage and can be preserved at -30 ~ -15°C for 3 months. Avoid repeated freeze-thaw cycles.

05/Test Method

05-1/Test Preparation

1. Take out the kit from the refrigerator and equilibrate it at room temperature (18 ~ 25°C) for at least 30 minutes.
2. Preparation of working wash buffer: Dilute the concentrated wash buffer (20×) with deionized water or distilled water by the dilution ratio of 1:19, and mix it well for later use. For example, use 30 ml of the concentrated wash buffer (20×) to prepare 600 ml of working wash buffer.
3. Preparation of enzyme-labeled detection antibody working solution: Add 100 µl of the enzyme-labeled detection antibody (100×) to 9.9 ml of the enzyme-labeled detection antibody diluent. Mix the solution well by shaking it upside down at least 30 times to prepare the enzyme-labeled detection antibody working solution.
   Notes: The enzyme-labeled detection antibody working solution can be stored at 2 ~ 8°C for same-day use. The volume of the working solution can be determined according to the sample size. 100 µl of enzyme-labeled detection antibody working solution is required for each sample or control.
4. Dilution of test sample:
   First test: Dilute the test sample with the sample diluent by the ratio of 1:40 and then by the ratio of 1:20. Use the diluted test sample for loading as the first batch of test wells and make a series of three-fold dilutions to obtain a total of 7 gradient concentrations.
   Subsequent analysis: According to the results of the first test, select the appropriate dilution factor or serial dilution for further analysis of sample reactivity.
5. Adjust the thermostatic incubator temperature to 37°C and use it after the temperature is stable.

05-2/Test Operation

1. Sample loading: Fix the pre-coated microplate strips on the plate rack. For each test, design 3 negative control wells and 2 positive control wells. The negative control wells are spaced, with at least three wells, apart from the positive control wells. Add 100 µl of the corresponding solution to each control well and 100 µl of the diluted test sample to each test well.
2. Incubation: Seal the plate with a sealing film and incubate it at 37°C for 60 minutes.
3. Washing: Remove the liquid in the wells, add at least 300 µl of the diluted wash buffer to each well, let the plate stand for 30 seconds, and discard the wash buffer in the wells. Repeat washing five times and pat the plate dry.
4. Enzyme loading: Add 100 µl of the enzyme-labeled detection antibody working solution to each well.
5. Incubation: Seal the plate with a sealing film and incubate it at 37°C for 30 minutes.
6. Washing: Remove the liquid in the wells, add at least 300 µl of the diluted wash buffer to each well, let the plate stand for 30 seconds, and discard the wash buffer in the wells. Repeat washing five times and pat the plate dry.
7. Color development: Add 100 µl of the TMB substrate solution to each well, seal the plate with a sealing film, and incubate it away from light at 37°C for 10 minutes.
8. Termination: Add 50 µl of stop solution to each well to terminate the reaction.
9. Reading: Use the microplate reader to measure the OD values of each well at the wavelengths of 450 nm and 630 nm.

06/Result Interpretation

1. Normal range of negative controls: The A values (OD value at 450 nm - OD value at 630 nm) of negative control wells should be ≤ 0.15. (If there is only one negative control well with an A value greater than 0.15, discard this well; if there are two or more negative control wells with an A value greater than 0.15, repeat the test.) The mean A value of positive control replicate wells should be greater than 0.8. If the A values of the negative control wells are < 0.05, they should be taken as 0.05 for calculation.
2. Calculation of the cut-off value:
   Cut-off value = Mean A value of negative controls × 2.1
3. Qualitative detection
   For the test sample diluted by 800-fold, if the A value ≥ cut-off value, the test sample is positive for the IgG antibody.
   For the test sample diluted by 800-fold, if the A value < cut-off value, the test sample is negative for the IgG antibody.

4. Titer determination

   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.

07/Performance Indicators

Precision: coefficient of variation (CV) < 15%

08/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 taken out of the refrigerator to room temperature before unpacking it for use, and shake the reagent well before use.
4. After the pre-coated microplate is unpacked, seal the remaining plate strips in an aluminum foil ziplock bag and then the packaging bag to avoid exposure to dampness.
5. Do not use kit components with different lot numbers interchangeably.
6. Do not use micropipette tips interchangeably to avoid cross-contamination.
7. If crystals appear in the concentrated wash buffer (20×), place the buffer at 37°C until crystals are dissolved before use.
8. Fill up each well with wash buffer during washing to ensure the residual reagent in each well is removed.
9. Read the result within 15 minutes after the reaction terminates.
10. The test results obtained with this product are for scientific research only.