Chicken Mycoplasma Gallisepticum Antibodies ELISA Kit

MATERIALS PROVIDED & STORAGE CONDITIONS

Item	Specification
ELISA Microtiter plate	96 wells
Dilution Plate	96 wells
HRP Conjugate	12 mL
10×Concentrated Wash Buffer	50 mL
Substrate Reagent A	6 mL
Substrate Reagent B	6 mL
Sample Diluent	50 mL
Stop Solution	6 mL
Positive Control	0.8 mL
Negative Control	0.8 mL
Plate Sealer	3 pieces
Sealed Bag	1 piece
Manual	1 copy

* Provided this is within the expiration date of the kit.

Test principle

This kit is comprised by HRP conjugate, other reagents and ELISA Microtiter plate pre-coated with recombinant chicken mycoplasma gallisepticum (MG) protein. Apply the principle of enzyme-linked immunoassay (ELISA) to detect MG antibody in serum of chicken. During the experiment, add control serum and samples into the ELISA Microtiter plate. If MG antibodies exist in the samples, it will be bound with the recombinant protein on the microtiter plate after incubation. Then wash the plate to remove unbound antibodies and other components, add the HRP conjugate to specifically bind with the compound of antibody and antigen on the microtiter plate. The unbound HRP conjugate will be removed by washing. Substrate Reagent is added into the well, it will react with the enzyme and the products become blue. The color shade is positive correlation with antibody levels in the samples. At last, end the reaction by adding stop solution to produce a yellow product. Measure the absorbance value of each well by using a Microplate Reader with 450 nm wavelength, then we can know whether there is chicken MG antibody in the samples.

Other materials required but not supplied

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

High-precision transferpettor, EP tubes and disposable pipette tips

37℃ incubator or water bath

Deionized or distilled water

Absorbent paper

Notes

1. Wear gloves and work clothes during experiment, and the disinfection and isolation system should be strictly performed. All the waste should be handled as contaminant.
2. The stop solution is corrosive, it should be avoided to contact with skin and clothing. Wash immediately with plenty of water if contacted carelessly.
3. FOR RESEARCH USE ONLY. ELISA Microtiter plate should be covered by plate sealer. Avoid the kit to stro ng light.
4. Concentrated wash buffer at low temperature condition is easy to crystallize, it should be adjusted to room temperature in order to dissolve completely before use.
5. Each well must be filled with liquid when washing in order to prevent residual free enzyme.
6. The tested sample should keep fresh.
7. The results shall depend on the readings of the microplate reader.
8. **Each reagent is optimized for use in the JN927018. Do not substitute reagents from any other manufacturer into the test kit. Do not combine reagents from other JN927018 with different lot numbers.**
9. If the samples are not indicated in the manual, a preliminary experiment to determine the validity of the kit is necessary.

Storage and expiry date

Store at 2-8℃. Avoid freeze.

Please store the opened plate at 2-8℃, the shelf life of the opened kit is up to 1 month.

Expiry date: expiration date is on the packing box.

Sample preparation

1. **Serum:** Use the conventional method to prepare serum, the serum must be clear, no hemolysis and no pollution. Samples can be conserved at 2-8℃ in 1 week, and it should be stored at -20℃ for a long-term storage.
2. **Diluted serum:** Dilute the sample serum with the **Sample Diluent** at 1:100 (2 µL sample serum and 200 µL of sample diluent, mix fully). The positive/negative control do not need to be diluted.
3. **Wash Buffer:** The **10×Concentrated Wash Buffer** should be adjusted to room temperature to make the sediment dissolved fully before use, then dilute it with deionized water at 1:9.

Assay procedure

Restore all reagents and samples to room temperature (25℃) before use. All the reagents should be mixed thoroughly by gently swirling before pipetting. Avoid foaming. The unused ELISA Microtiter plate should be sealed as soon as possible and stored at 2-8℃.

1. **Number:** number the sample and control in order (multiple well), and keep a record of control wells and sample wells. Set 1 well for blank/negative/positive control respectively. **Samples need test in duplicate.**
2. **Add sample:** add 100 µL of **positive/negative control** to positive/negative control well, add 100 µL of **Sample Diluent** to blank control well and add 100 µL of **Diluted serum** to the sample wells.
3. **Incubate:** cover the plate sealer and mix thoroughly, incubate at 37℃ for 30 min in shading light.
4. **Wash:** remove the liquid in each well. Immediately add 300 µL of **Wash Buffer** to each well and wash. Repeat wash procedure for 3 times, 1 min intervals/time. Invert the plate and pat it against thick clean absorbent paper (If bubbles exist in the wells, clean tips can be used to prick them).
5. **HRP conjugate:** add 100 µL of **HRP Conjugate** into each well, cover the plate sealer and incubate at 37℃ for 30 min in shading light.
6. **Wash:** repeat step 4 for washing.
7. **Color Development:** add 50 µL of **Substrate Reagent A** and 50 µL of **Substrate Reagent B** into each well. Cover the plate sealer and mix thoroughly, incubate at 37℃ for 10 min in shading light.
8. **Stop reaction:** add 50 µL of **Stop Solution** into each well, mix thoroughly.

9. **OD Measurement:** measure the absorbance value (A-value) of each well by using a Microplate Reader with 450 nm/630 nm wavelength. This step should be finished in 10 min after stop reaction.

Reference value

Normally, the A-value of negative control < 0.15 and positive control ≥ 0.4.

Interpretation of the results

1. Cut Off (C.O) =0.25+Average of negative control ODs (when Average of negative control ODs < 0.05, it is calculated as 0.05.)
2. Positive result: Sample OD > Cut Off (C.O)
3. Negative result: Sample OD < Cut Off (C.O)
4. Unimmunized animal: positive result indicates that it may be infected with MG.

Immunized animal: The antibody levels at the time of the sample were monitored and recorded, and the distribution of antibody levels and the trend of immune status of the flock were analyzed based on the results.

Limitations of this test method

1. This test is only used as the qualitative detection of MG antibodies in serum of chicken. A rough estimate of antibody concentration (high, general, low) can be calculated based on the OD value.
2. The detection results of this kit are only for reference. For confirmation of the result, please combine the symptoms and other methods of detection, this detection cannot be used as the only criteria for result