Chicken Infectious Bursal Disease Antibodies ELISA Kit

Catalog No: E-AD-E061

96T/96T*2/96T*5

Version Number:	V1.2
Replace version:	V1.1
Revision Date:	2024.03.14

This manual must be read attentively and completely before using this product.

If you have any problems, please contact our Technical Service Center for help.

Toll-free: 1-888-852-8623 Tel: 1-832-243-6086 Fax: 1-832-243-6017
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Please kindly provide us the lot number (on the outside of the box) of the kit for more efficient service.

Test principle

This kit is comprised by HRP conjugate, other auxiliary reagents, ELISA Microtiter plate pre-coated with the Infectious Bursal Disease (IBD) VP2 antigen. Apply the principle of enzyme-linked immunoassay (ELISA) to detect IBD antibody in serum of chicken. During the experiment, add control serum and samples into the ELISA Microtiter plate. If IBD antibodies exist in the samples, it will compete with the antibody in the antibody working solution to bind with the antigen pre-coated on the Microplate. Then wash 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 become blue. The color shade is negative correlation with IBD 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 judge whether IBD antibody exist in the sample.

Kit components

Item	Specification
ELISA Microtiter plate	96 wells
101×Concentrated Antibody Working Solution	110 μL
10×Concentrated Wash Buffer	50 mL
HRP Conjugate	12 mL
Antibody Working Diluent	12 mL
Substrate Reagent A	6 mL
Substrate Reagent B	6 mL
Stop Solution	6 mL
Positive Control	50 μL
Negative Control	50 μL
Plate Sealer	3 pieces
Sealed Bag	1 piece
Manual	1 copy

Note: All reagent bottle caps must be tightened to prevent evaporation and microbial pollution.

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 th e kit to strong 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 E-AD-E061. Do not substitute reagents from any other manufacturer into the test kit. Do not combine reagents from other E-AD-E061 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 and reagents 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. **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.
3. ***Please calculate the amount of Antibody Working Solution based on the number of samples.*** **Antibody Working Solution:** The **101×Concentrated Antibody Working Solution** should be adjusted to room temperature before use, then dilute it with **Antibody Working Diluent** at 1:100. *Prepare the fresh solution before use.*

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 negative/positive control respectively and 1 well for blank control respectively. **Samples need test in duplicate**.
2. **Add sample:** add 10 μL of **positive/negative control** to positive/negative control well, add 10 μL of **Serum** to sample well. Then add 90 μL of **Antibody Working Solution** to each well, add only 100 μL of **Antibody Working Solution** to blank control.
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 wavelength (use 630 nm as reference wavelength). This step should be finished in 10 min after stop reaction. Blank well is not necessary for dual-wavelength detection

Reference value

Normally, the A-value of positive control /A-value of negative control < 0.7.

Analysis of results

1. S/N value = Sample OD/Average of negative control ODs.
2. Negative result : S/N value ≥ 0.7
3. Positive result : S/N value < 0.7
4. Unimmunized animal: positive result indicates that it may be infected with IBD.
5. 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 IBD antibodies in serum of chicken.
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.