Science for Society

Avian Infectious Bursal Disease (IBD) virus VP2 antibody

INSTRUCTION MANUAL

FOR PRODUCT No: LT41003AYSL

Arsh Biotech Pvt. Ltd.

308, Aggarwal City Mall, Road No.44,
Pitampura, Delhi-110034, India
Toll Free: 1800-2745-745
Mobile: +91-98105-21400 | Fax: +91-11-42208444
info@arshbiotech.com

MANUAL VERSION 1.01

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INTRODUCTION

The Avian Infectious Bursal Disease virus VP2 antibody (IBDV-VP2 Ab) ELISA kit is developed to detect the IBDV-VP2 antibodies level in chicken serum sample and can be used to evaluate Infectious Bursal Disease vaccine status in chickens.

PRINCIPLE

This kit is based on solid-phase enzyme-linked immunosorbent assay (ELISA) principle, composed by the reaction Micro-plate coated with high purity IBDV-VP2 antigen, horseradish peroxidase-labeled anti-chicken IgG and other reagents. The reaction mechanism is the coated antigen binding with IBDV-VP2-Ab in sample, and then with the enzyme-labeled anti-chicken IgG antibody to form a "coated antigen + IBDV-VP2-Ab + anti-chicken IgG HRP antibody" complex, add substrate, it will have coloration by the enzyme catalytic reaction. Color depth is proportional to the amount of IBDV-VP2-Ab, when the sample chromogenic reaction, the results detected by the microplate reader exceeds a set threshold value result judged as positive, indicating that the immune produce antibodies or natural infection exists.

MATERIALS SUPPLIED WITH THIS KIT

1	IBDV-VP2 antigen coated microplate	96T X 2	6	Substrate	12ml X2 orange lid
2	Enzyme conjugate	22ml yellow lid	7	Stop solution	12ml blue lid
3	Sample diluent	50ml transparent lid	8	20×concentrated washing buffer	50ml white lid
4	IBDV-VP2-IgG Negative control serum	1.5ml green lid	9	Adhesive Foil	2 pieces
5	IBDV-VP2-IgG Positive control serum	1.5ml red lid	10	Instruction	1 piece

MATERIALS REQUIRED BUT NOT PROVIDED

1) Microplate Reader (double-wave length: 450/630 nm).

2) Precise micropipette (single-channel 1-100µl, 0.5-10µl, multi-channel 30-300µl).

3) Constant temperature box or water bath.

4) Oscillator.

5) Microplate Washer.

6) Disposable tips (10µl, 200µl).

7) Deionized water.

SAMPLE REQUIREMENT

1. The test sample is chicken serum, collecting sample without bacteria, store at 2 ℃～8℃ for less than a week, store at lower than -20℃ for long-term storage.

2. Avoid using sample of severe hemolysis, sediments, containing suspended long f ibrin and pollution bacteria.

3. Samples with conventional dosage of EDTA, sodium citrate or sodium heparin a nticoagulant do not affect the experiment.

PREPARATION

1. Bring ELISA reagents to the room temperature (20-25 ℃) for 30 min to get best results.

2. Sample dilution: use the sample diluent at 40 times. Mix the diluted sample evenly to get better results.

3. Washing solution preparation: Dilute the 20×concentrated washing buffer with deionized water at 20 times.

PROCEDURE

1. Adding sample: Take out the required coated plates according to sample quantit y (Can be detached) and record the sample position on a worksheet. Set 2 wells for negative control serum and 2 wells for positive control serum, add undiluted negati ve and positive control serum to its well accordingly, 100 µl/well. Others are sampl e wells, add the diluted sample, 100 µl/well (both single-well and double-well test is OK).

2. Incubation: cover with Adhesive Foil after adding sample, **incubate at 37℃ for 30 min**.

3. Remove adhesive foil. Pour the liquid out of the wells, add the diluted Washing solution into each well fully, no need to be static, pour.

4. Add 100µl enzyme conjugate into each well.

5. Cover plate with new adhesive foil. **Incubate at 37 ℃ for 30 min**.

6. Repeat step 3 (washing).

7. Add 100µl substrate into each well, mix properly, **incubate for 10 min at 37 ℃ in the dark** with new adhesive foil.

8 Add 50µl stop solution into each well, mix gently and determine the result.

9 Measure the OD value of each well with a photometer at dual-wave length 450nm/630nm.

RESULTS

For the assay to be valid, the positive control wells average OD value must be greater than or equal to 0.6, and the negative control wells average OD value is less than 0.15. Otherwise the test is invalid, needs to be tested again.

The result is judged by S/P value,

S/P = (Sample OD450/630- $NC\bar{x}$)/($PC\bar{x}$- $NC\bar{x}$), $NC\bar{x}$ means Negative control's average OD450/630 value, $PC\bar{x}$ means Positive control's average OD450/630 value If S/P≥0.20, it is positive; less than 0.20, it is negative.

INTERPRETATION OF THE RESULT

1. Sevre hemolysis, fiber protein in the serum separation is not sufficient, containing erthyrocytes, a precipitate, a sample with bacteria may lead to false positive.
2. Negative results may occur on individual chicken after vaccines due to individual differences or immune duration.
3. Positive results for serological diagnosis and epidemiological investigation of chicken to be combined with other methods and clinical data.

PRODUCT PERFORMANCE

1. Specificity: use this kit to detect reference serum, the compliance rate reach 100%.
2. Sensitivity: can reach max 1:12800.
3. Precision: CV (%) no bigger than 8%.

PRECAUTIONS AND WARNINGS FOR USERS

1. This test kit is suitable for *in vitro* diagnostics.
2. Do not use expired reagents, do not mix reagents from different lots.
3. Read the Manual carefully before use.
4. Experiment rubbish should be dealt with high pressure steam sterilization at 121 ℃ for 30 minutes, or treated with 5.0g/L sodium hypochlorite disinfectant for 30 minutes, then discard.

5. MicroWell plate removed from the refrigerated environment should be balanced moisture to dry at room temperature, then can be opened. Put back unused MicroWell plate into dry foil bag and sealed at 4 ℃. Unused liquid reagent should cover caps, store at 2-8 ℃ in dark with other group components.

6. If the 20×concentrated washing buffer appears crystal, it is normal, put at 37℃ until been dissolved.

7. Should use Micropipettor to add sample and reagents, and often proof its accuracy.

8. When adding washing buffer, should be full but no overflow, avoid appearing free enzyme at mouth of well or cross pollution between wells.

9. Stop solution is corrosive, use large amount of water to wash immediately when touch the skin or clothes.

Specifications: 96 wells×2.

Expiry date: 12 months.

Storage: Store at 2~8℃, in the dark.