VERSION 1.01

INFECTION BRONCHITIS VIRUS (IBV) ANTIBODY ELISA KIT

MANUAL

ELISAKITS.ONLINE
By Immunomart

Infectious Bronchitis Virus (IBV) Antibody ELISA Kit

Catalogue Number. IP100191

Introduction

The Infectious Bronchitis IgG (IBV) antibody ELISA kit is developed to detect the IBV antibodies level in chicken serum sample and can be used to evaluate serological diagnosis of infected chickens and Epidemiological surveys of Infectious Bronchitis virus, analysis of Infectious Bronchitis virus vaccine status in chickens.

Principle

The Infectious Bronchitis IgG (IBV) antibody ELISA kit is developed to detect the IBV antibodies level in chicken serum sample and can be used to evaluate serological diagnosis of infected chickens and Epidemiological surveys of Infectious Bronchitis virus, analysis of Infectious Bronchitis virus vaccine status in chickens.

Specifications: 96 wells × 2.

Components

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

Material required not provided

1. Micropipettes: 0.5μL~10μL、10μL~100μL、100μL~1000μL
2. Disposable pipette tips
3. Measuring cylinder: 500 ml
4. 96 wells microplate reader

5. Distilled water or deionized water
6. Bottle or microplate washing machine

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 fibrin and pollution bacteria.
3. Samples with conventional dosage of EDTA, sodium citrate or sodium heparin anticoagulant do not affect the experiment**.**

Preparation

1) Bring ELISA reagents to the room temperature (20-25 ℃) for 30 min to get best results.
2) Dilute sample with sample diluent at 40 times (there is color change after adding sample), mix diluted sample evenly can get better result.
2) Washing solution preparation: Dilute the 20×concentrated washing buffer with deionized water at 20 times**.**

Operation procedures

1 .Adding sample: Take out the required coated plates according to sample quantity (Can be detached) and record the sample position on a worksheet. For single-wave length test, set one blank control well, add nothing; for double-wave length test, do not set blank control well. Set 2 wells for negative control serum and 2 wells for positive control serum, add undiluted negative and positive control serum to its well accordingly, 100 µL/well. Others are wells for samples, add 100µL/well of Sample diluent solution, then add 1 µL serum sample separately (there will be color change after adding sample).
2. Mix gently for 30s, incubate at 37℃ for 30 min.
3. Remove adhesive foil. Pour the liquid out of the wells, add Washing solution (dillute the Wash Concentrate at 20 times with deionized water) into each well fully, stand for 1 min. Repeat 5 times, at last time pat to dry on absorbent paper.
4. Add 100 µL enzyme conjugate into each well (except the blank well, also do not add any liquid to blank well).
5. Cover plate with new adhesive foil. Incubate at 37 ℃ for 30 min.
6. Repeat step 3(washing).
7. Add 50µL substrate A and substrate 50µL B into each well, mix properly, incubate for 15 min at 37 ℃ in the dark with new adhesive foil.
8. Add 50µL stop solution into each well, mix gently and determine the result within 5-30 min.
9. For single-wavelength test, measure the A value with a photometer at 450 nm, set zero for the blank well, and read A value of each well; For double-wavelength test, measure the A value with a photometer at 450 nm (Reference-wavelength: 630 nm), read A value of each well.

Results

Generally speaking, the IBV-Positive control average OD value should be ≥ 0.6, the IBV-Negative control average OD value should be less than 0.1, otherwise the experiment do not success, re-test it.
The result is judged by S/P value,
S/P=(Sample OD450/630- NCx(—))/(PCx(—)- NCx(—)), NCx(—) means Negative control's average OD450/630 value, PCx(—) means Positive control's average OD450/630 value

Interpretation of the result

1. Severe hemolysis, fiber protein in the serum separation is not sufficient, containing erythrocytes, 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 1:10240.
3. Precision: CV（%）no bigger than 8%.
4. Stability: Store at 2℃～8℃ for 12 months or store at 37℃ for 3 days, the result can reach the above 3 standards. If S/P≥0.2, it is positive; less than 0.2, it is negative.

Precautions

1. This test kit is suitable for in vitro diagnostics.
2. Do not use kits out of expiry date, do not mix use reagents from different lots.
3. Read the manual carefully before using the kits.
4. Wear glove and working clothes when operate, treat the test kit as containing infectious material. 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 2-8 ℃. 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.

storage: store at 2-8℃, dark, sealed, dry place, no frozen..
Expiry date: 12 months; date of production is on box.