General Information

This indirect ELISA diagnostic kit is designed to detect antibodies directed against the Infectious Bronchitis virus (IBV).

It is a quantitative test for the detection of IBV-specific antibodies in chicken serum, plasma or egg yolk samples.

For use in other species, please contact us.

Description and Principle

Microwells are coated with purified IBV recombinant protein.

Samples to be tested and controls are added to the wells. Anti-IBV antibodies, if present, form an antigen-antibody complex.

After washing, an anti-chicken horseradish peroxidase (HRP) conjugate is added to the wells. It fixes to the antibodies, forming an antigen-antibody-conjugate-HRP complex.

After elimination of the excess conjugate by washing, the substrate solution (TMB) is added.

The resulting coloration depends on the quantity of specific antibodies present in the specimen to be tested:

- in the presence of antibodies, a blue coloration appears which becomes yellow after addition of the stop solution.
- in the absence of antibodies, no coloration appears.

The microplate is read at 450 nm.

Kit Components

Reagents*
Microplates coated with purified IBV recombinant protein
Positive Control
Negative Control
Concentrated Conjugate (10X)
Dilution Buffer 3
Dilution Buffer 14
Wash Concentrate (20X)
Substrate Solution
Stop Solution (0,5 M)

** Quantities supplied are indicated on the kit label.*

1. The conjugate, the controls and the substrate solution must be stored at 5°C (± 3°C).
2. Other reagents can be stored between +2°C and +26°C.
3. For detailed storage conditions of opened and/or diluted components, please refer to www.innovative-diagnostics.com/storage-conditions/
4. Wash and stop solutions can be used for the entire IDvet product range. Substrate solutions and dilution buffers with same batch numbers are interchangeable.

Materials required but not provided

1. Mono or multi-channel pipettes capable of delivering volumes of 5 µl, 10 µl, 100 µl, and 500 µl.
2. Disposable tips.
3. 96 well pre-dilution plate.
4. Distilled or deionized water.
5. Manual or automatic wash system.
6. 96-well microplate reader.

Precautions

1. Do not pipette by mouth.
2. Contains components that can be harmful to the skin and eyes and may cause sensitisation by skin contact. Avoid contact with skin and eyes. Use protective lab coat, one-way gloves and safety glasses. The stop solution (0,5 M acid) may be harmful if swallowed.
3. Do not expose the substrate solution to bright light nor to oxidizing agents.
4. All waste should be properly decontaminated prior to disposal. Dispose in accordance with local regulations.

Please refer to the Material Safety Data Sheet, available upon request at info@innovative-diagnostics.com, for more detailed information

Wash Solution Preparation

If necessary, bring the Wash Concentrate **(20X)** to room temperature and mix thoroughly to ensure that the Wash Concentrate **(20X)** is completely solubilized.

Prepare the Wash Solution **(1X)** by diluting the Wash Concentrate **(20X)** to 1:20 in distilled/deionized water.

The quality of the wash step may influence results. Ensure that wells are completely empty between washes. If using an automatic washer, it is extremely important to correctly parameter the machine (mode, type of aspiration, aspiration height). For more information, please consult the "IDvet Washing Guide", available upon request.

Testing Procedure

Allow all reagents to come to room temperature 21°C (± 5°C) before use. Homogenize all reagents by inversion or vortexing.

The negative and positive controls are supplied ready-to-use. DO NOT add dilution buffer to the control wells A1, B1, C1 and D1 – controls are to be tested un-diluted. Samples, however, are tested at a **final dilution of 1:500**.

For serum or plasma samples (1:50 pre-dilution, followed by 1:10 dilution in the ELISA microplate):

1. In a pre-dilution plate, set aside wells A1, B1, C1 and D1 for the controls, and add:
   - 5 µl of **each sample to be tested**,
   - 245 µl of **Dilution Buffer 14** to all wells EXCEPT to control wells A1, B1, C1 and D1.

Note: It is recommended to respect the indicated order of deposit to be able to visually control addition of sample to each well.

2. In the ELISA microplate, add:
   - 100 µl of the **Negative Control** to wells A1 and B1.
   - 100 µl of the **Positive Control** to wells C1 and D1.
   - 90 µl of **Dilution Buffer 14** to as many wells as there are samples to be tested (NOT to control wells A1, B1, C1 and D1).
   - 10 µl of each **pre-diluted serum/plasma sample** as prepared above.
3. Cover the plate and incubate **30 min** ± **3 min** at 21°C (± 5°C).

For egg yolk samples (1:5 and 1:50 pre-dilutions, followed by 1:2 dilution in the ELISA microplate):

1. a) Pre-dilute the samples as follows:
   - Mix 80 µl of **Wash Solution 1X** with 20 µl of **egg yolk,**
   - Mix well before testing by inversion or vortexing.

Note: These diluted egg yolk samples may be stored at -20°C for future testing. Samples may not undergo more than 3 freeze-thaw cycles.

1. b) In a pre-dilution plate, set aside wells A1, B1, C1 and D1 for the controls, and add:
   - 5 µl of **each sample to be tested**,
   - 245 µl of **Dilution Buffer 14** to all well EXCEPT to control wells A1, B1, C1 and D1.

Note: It is recommended to respect the indicated order of deposit to be able to visually control addition of sample to each well.

2. In the ELISA microplate, add:
   - 100 µl of the **Negative Control** to wells A1 and B1.
   - 100 µl of the **Positive Control** to wells C1 and D1.
   - **50 µl** of **Dilution Buffer 14** to as many wells as there are samples to be tested (NOT to control wells A1, B1, C1 and D1).
   - 50 µl of each **pre-diluted egg yolk sample** as prepared above.

Warning: *Make sure that the 50 + 50 µl mixture is homogeneous to avoid an increase of the background signal which could generate false positive results. In case of doubt, it is possible to realize the mixture, outside the plate, in tubes and then vortex before adding the 100 µl in the ELISA plate.*

3. Cover the plate and incubate **30 min** ± **3 min** at 21°C (± 5°C).

For all sample types:

4. Prepare the **Conjugate 1X** by diluting the **Concentrated conjugate 10X** to 1:10 in **Dilution Buffer 3.**
5. Empty the wells. Wash each well 3 times with at least 300 µl of the **Wash Solution 1X.** Avoid drying of the wells between washes.
6. Add 100 µl of the **Conjugate 1X** to each well.
7. Cover the plate and incubate **30 min** ± **3 min** at 21°C (± 5°C).
8. Empty the wells. Wash each well 3 times with a least 300 µl of the **Wash Solution 1X.** Avoid drying of the wells between washes.
9. Add 100 µl of the **Substrate Solution** to each well.
10. Cover the plate and incubate **15 min** ± **2 min** at 21°C (± 5°C) in the dark.
11. Add 100 µl of the **Stop Solution** to each well in the same order as in step No. 9 to stop the reaction.
12. Read and record the O.D. at 450 nm.

Validation

The test is validated if:

✓ the mean OD value of the Positive Control (OD_{PC}) is greater than 0.250.

$$\mathbf{OD_{PC} > 0.250}$$

✓ the ratio of the mean values of the Positive and Negative Controls (OD_{PC} and OD_{NC}) is greater than 3.

$$\mathbf{OD_{PC}/OD_{NC} > 3}$$

Interpretation

For each sample, calculate the S/P ratio and antibody titer as follows:

S/P ratio

$$\mathbf{S/P = \frac{OD_{sample} - OD_{NC}}{OD_{PC} - OD_{NC}}}$$

Antibody titer

$$\mathbf{\log_{10}(titer) = 0.7 \times \log_{10}(S/P) + 3.7}$$

$$\mathbf{titer = 10^{\log_{10}(titer)}}$$

Results are interpreted as follows:

S/P value	ELISA Antibody titer	IBV Immune status
S/P ≤ 0.2	Titer ≤ 1625	Negative
S/P > 0.2	Titer > 1625	Positive

Note: The IDSoft™ data analysis program is available free-of-charge. For more information, please contact support.software@innovative-diagnostics.com.

This software program can calculate many parameters (validation criteria, S/P values, titers, vaccination age, groups) and offers a graphic representation of the serological profiles of the animals tested.

Certified management system

ID Screen®
Infectious Bronchitis Indirect 2.0

Indirect ELISA for the detection of antibodies against IBV in chicken serum, plasma or egg yolk samples.

For *in vitro* use

February 2023

New antibody titer formula and associated cut-off titer value (titer >1625 instead of 853)

0014110

IBVARSV2 ver 0223 EN

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