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flocktype® IBV Ab Handbook

2 (catalog no. 274302)

5 (catalog no. 274303)*

For the detection of antibodies to infectious bronchitis virus

REF 274302, 274303*

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* Available only on request.

Sample & Assay Technologies

QIAGEN Sample and Assay Technologies

QIAGEN is the leading provider of innovative sample and assay technologies, enabling the isolation and detection of contents of any biological sample. Our advanced, high-quality products and services ensure success from sample to result.

QIAGEN sets standards in:

- Purification of DNA, RNA, and proteins
- Nucleic acid and protein assays
- microRNA research and RNAi
- Automation of sample and assay technologies

Our mission is to enable you to achieve outstanding success and breakthroughs. For more information, visit www.qiagen.com.

In addition, QIAGEN provides high-quality, easy-to-use, and sensitive molecular solutions to enable veterinary pathogen detection and animal pathogen research. The QIAGEN veterinary portfolio includes a broad range of pathogen-specific PCR-assays and an extensive and growing ELISA portfolio. For more information, visit www.qiagen.com/Animal-and-Veterinary-Testing.

Contents

Kit Contents

***flocktype* IBV Ab**		
Catalog no.	**274302**	**274303***
Number of plates	**2**	**5**
Test Plate: microtiter plate with 96 wells, coated with non-infectious IBV antigen	2	5
Sample diluent, ready-to-use	1 x 125 ml	2 x 125 ml
Negative Control, ready-to-use	1 x 3.5 ml	1 x 3.5 ml
Positive Control, ready-to-use	1 x 3.5 ml	1 x 3.5 ml
Wash buffer (10x)	1 x 125 ml	2 x 125 ml
Conjugate, ready-to-use	1 x 24 ml	1 x 60 ml
TMB substrate, ready-to-use	1 x 24 ml	1 x 60 ml
Stop solution, ready-to-use	1 x 24 ml	1 x 60 ml
Handbook	1	1

* Available only on request.

Intended Use

The *flocktype* IBV Ab is a specific and sensitive ELISA for detecting antibodies to infectious bronchitis virus (IBV) in serum and plasma samples from chickens. For veterinary use only.

Symbols

Contains reagents for <N> plates


Legal manufacturer

Lot number


Use by date


Temperature limitations for storage

Handbook

Catalog number

Material number


Protect from light


For chicken samples

Storage

The components of the *flocktype* IBV Ab ELISA should be stored at 2–8°C and are stable until the expiration date stated on the label. Wash Buffer (10x) and Stop Solution may be stored at room temperature (18–25°C) to avoid salt crystallization. If test strips are provided with the kit, store the remaining test strips in the re-sealed foil pouch with desiccant at 2–8°C until next use. The test strips can be stored for at least 6 weeks after opening the plate pouch.

Safety Information

When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, please consult the appropriate safety data sheets (SDSs). These are available online in convenient and compact PDF format at www.qiagen.com/safety where you can find, view, and print the SDS for each QIAGEN kit and kit component.

CAUTION: The Stop Solution contains 0.5 M sulphuric acid.

All sample residues and objects which have come into contact with samples must be decontaminated or disposed as potentially infectious material.

Quality Control

In accordance with QIAGEN's ISO-certified Quality Management System, each lot of *flocktype* IBV Ab is tested against predetermined specifications to ensure consistent product quality.

Introduction

The *flocktype* IBV Ab is a highly sensitive and specific solution for the detection of antibodies to infectious bronchitis virus (IBV) in serum and plasma samples from chickens.
Infectious bronchitis (IB) is usually characterized by respiratory symptoms and of economically importance for the poultry industry. The detection of antibodies against IBV using *flocktype* IBV Ab is a reliable method to monitor humoral vaccination responses or IBV infections. The *flocktype* IBV Ab uses the nucleocapsid protein of the virus prepared by recombinant technology as antigen. This structural protein is highly conserved and immunogenic.
The *flocktype* IBV Ab in combination with the FlockSoft™ software is capable of calculating the antibody titer in chicken and of quantitatively depicting the results.

Principle

The microtiter plate is coated with a recombinant structural protein from the virus. During sample incubation IBV-specific antibodies bind to the immobilized antigen. Unbound material is removed by rinsing. The anti-IgY-HRP conjugate detects serum antibodies bound to the antigen. Unbound conjugate is removed by rinsing. A colorimetric reaction is initiated by adding Substrate Solution and stopped after 10 minutes. In the presence of IBV-specific antibodies, within the sample, HRP catalyzes a blue color development, which turns yellow after adding the Stop Solution. The optical density (OD) is measured in a spectrophotometer. The OD values correlate with the concentration of anti-IBV antibodies in the sample.

Equipment and Reagents to Be Supplied by User

When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, consult the appropriate safety data sheets (SDSs), available from the product supplier.

- Beakers
- Measuring cylinders
- Pipets (adjustable)
- Multichannel pipets (adjustable)
- Aluminum or adhesive foil for covering the Test Plate
- Device for delivery and aspiration of wash solution (optional)
- Microtiter plate absorbance reader
- Tubes or plates for diluting the samples
- Distilled water

General precautions

The user should always pay attention to the following:

- Do not expose the TMB Substrate Solution to intense light or to sunlight during the performance of the test.
- Components of the test kit should not be contaminated or mixed with components from other batches.
- Do not use the components of the test kit past expiration date.
- Water from ion-exchange systems used for diluting the Wash Solution (10x) may interfere with the assay if not pure enough. Water quality of double distilled water or highly purified water (Milli-Q) is suitable.
- The use of clean glass devices, careful pipetting and rinsing during the test, and strict adherence to the indicated incubation times is essential for precise test results.

Things to do before starting

- Bring reagents to room temperature (18-25°C) immediately before use. In case of precipitated salt crystals in the Wash Buffer (10x), dissolve by gentle swirling and warming.

Wash Buffer: Dilute Wash Buffer (10x) 1:10 in distilled water, for example, for one test plate dilute 25 ml Wash Buffer (10x) in 225 ml distilled water and mix.

Serum/plasma: Prior to sample analysis, with serum/plasma samples, dilute **1:500** in Sample Diluent (e.g., dilute 1 µl sample in 499 µl Sample Diluent) and mix well. Use plastic tubes or uncoated microtiter plates for dilution. Change pipet tips for each sample.

Alternatively, serum/plasma samples can be diluted from a pre-dilution (1:50 in Sample Diluent) directly in the Test Plate (see Procedure step 1a).

- **Controls** are ready-to-use and do not require dilution.

Protocol: ELISA

Please read “Things to do before starting”, page 10.

Procedure

1. Pipet 100 µl of each of the ready-to-use Negative Control (in duplicates) and Positive Control (in duplicates) and the 1:500 samples into the Test Plate wells.

1a. Alternatively, pipet 90 µl of Sample Diluent in each sample well and add 10 µl of the of the 1:50 pre-diluted sample. Mix well.

   Record the positions of the controls and samples in a test protocol. The use of a multichannel pipet is recommended for the transfer of samples. Cover the Test Plate.

2. Incubate for 30 min at room temperature (18–25°C).
3. Remove solution from the wells by aspiration or tapping.
4. Rinse each well 3x with 300 µl of prepared Wash Buffer. Remove the buffer after each rinse.
5. Pipet 100 µl ready-to-use Conjugate to each well and incubate for 30 min at room temperature (18–25°C). Cover the Test Plate.
6. Remove solution from wells by aspiration or tapping.
7. Rinse each well 3x with 300 µl of prepared Wash Buffer. Remove the buffer after each rinse.
8. Pipet 100 µl TMB Substrate Solution to each well.
9. Incubate for 10 min at room temperature in the dark. Begin timing after the first well is filled.

10. Stop the reaction by adding 100 µl Stop Solution per well. Add the Stop Solution in the same order as the Substrate Solution was added.
11. Measure the OD in the plate reader at 450 nm within 20 min after stopping the reaction.

    Measuring at a reference wavelength (620–650 nm) is optional.

Data Interpretation

Validation criteria

The results are valid if the following criteria are met:

- The mean value (MV) of the measured OD value for the Positive Control (PC) must be ≥ 0.7
- The MV of the measured OD value for the Negative Control (NC) must be ≤ 0.2

In case of invalid assays the test should be repeated after a thorough review of the instructions for use.

Calculation

Calculate the MV of the measured OD for the NC and the PC.

The ratio (S/P) of sample OD to mean OD of the Positive Control is calculated according to the following equation:

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

Endpoint titers are calculated from the S/P ratio at a 1:500 dilution using the following equation:

$$Log_{10}\,Titer = 1.22\ (Log_{10}\,S/P) + 3.55$$

Interpretation of the results

Samples with the S/P ratio <0.2 are negative.
Specific antibodies to IBV could not be detected.

Samples with the S/P ratio ≥0.2 and <0.3 are doubtful.
Doubtful results should be grouped to the majority of the positive or negative results. It is recommended to retest doubtful results after a few weeks. Doubtful results from recently vaccinated animals may indicate the beginning of an increase in the formation of specific antibodies. Doubtful results from animals with repeated vaccinations may indicate an insufficient formation or a decrease of specific antibodies.

Samples with the S/P ratio ≥0.3 are positive.
Specific antibodies to IBV were detected.

Troubleshooting Guide

The scientists in QIAGEN Technical Services are always happy to answer any questions you may have about either the information and/or protocols in this handbook or sample and assay technologies (for contact information, see back cover or visit www.qiagen.com).

Ordering Information

Product	Contents	Cat. no.
flocktype IBV Ab (2)	For 192 reactions: 2 Test Plates (strips), Wash Buffer, Sample Diluent, Positive Control, Negative Control, Conjugate, TMB Substrate Solution, Stop Solution	274302
flocktype IBV Ab (5)*	For 480 reactions: 5 Test Plates, Wash Buffer, Sample Diluent, Positive Control, Negative Control, Conjugate, TMB Substrate Solution, Stop Solution	274303
Related products		
flocktype IBDV Ab (2)†	For 192 reactions: 2 Test Plates (strips), Wash Buffer, Sample Diluent, Positive Control, Negative Control, Conjugate, TMB Substrate Solution, Stop Solution	274202
flocktype NDV Ab (2)†	For 192 reactions: 2 Test Plates (strips), Wash Buffer, Sample Diluent, Positive Control, Negative Control, Conjugate, TMB Substrate Solution, Stop Solution	275002
flocktype Mycoplasma Mg Ab (2)*	For 192 reactions: 2 Test Plates (strips), Wash Buffer, Sample Diluent, Positive Control, Negative Control, Conjugate, TMB Substrate Solution, Stop Solution	274502

*Available only on request.
† Other kit sizes are available; see www.qiagen.com.

Product	Contents	Cat. no.
flocktype Mycoplasma Mg/Ms Ab (5)*	For 480 reactions: 5 Test Plates, Wash Buffer, Sample Diluent, Positive Control, Negative Control, Conjugate, TMB Substrate Solution, Stop Solution	274803
flocktype Mycoplasma Ms Ab (2)*	For 192 reactions: 2 Test Plates (strips), Wash Buffer, Sample Diluent, Positive Control, Negative Control, Conjugate, TMB Substrate Solution, Stop Solution	274602
flocktype AIV Ab (2)*	For 192 reactions: 2 Test Plates (strips), Wash Buffer, Sample Diluent, Positive Control, Negative Control, Conjugate, TMB Substrate Solution, Stop Solution	274012
flocktype Salmonella Ab (2)*	For 192 reactions: 2 Test Plates (strips), Wash Buffer, Sample Diluent, Positive Control, Negative Control, Conjugate, TMB Substrate Solution, Stop Solution	275702

* Other kit sizes are available; see www.qiagen.com.

QIAGEN offer a range of ELISA kits and real-time PCR and real-time RT-PCR kits for the detection of animal pathogens. Visit www.qiagen.com/Animal-and-Veterinary-Testing for more information about the *bactotype*®, *cador*®, *cattletype*®, *flocktype*, *pigtype*®, and *virotype*®.

For up-to-date licensing information and product-specific disclaimers, see the respective QIAGEN kit handbook or user manual. QIAGEN kit handbooks and user manuals are available at www.qiagen.com or can be requested from QIAGEN Technical Services or your local distributor.

Quick guide

Sample dilution:
Serum/plasma 1:500

Step	
1. Sample	100 µl/well
2. Incubation	30 min RT
3. Wash	3 x 300 µl
4. Conjugate	100 µl/well
5. Incubation	30 min RT
6. Wash	3 x 300 µl
7. TMB	100 µl/well
8. Incubation	10 min RT
9. Stop	100 µl/well
10. Read	450 nm

Data interpretation

Negative	Doubtful	Positive
S/P <0.2	S/P ≥0.2 and <0.3	S/P ≥0.3

Notes

Notes

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