December 2022 | EN

flocktype® AIV Ab
Handbook

For detection of antibodies to Avian Influenza A Virus

Licensed in accordance with § 11 (2) of the German Animal Health Act
MA No.: FLI-B 435

REF 2 plates (cat. no. FT274012)

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INDICAL BIOSCIENCE

Contents

Kit contents

flocktype AIV Ab **Cat. no.** **Number of plates**	**(2)** **FT274012** **2**
Test Plate: microtiter plate with 96 wells, coated with non-infectious AIV antigen	2
Sample Diluent, ready to use	1 x 125 ml
Negative Control, ready to use	1 x 3.5 ml
Positive Control, ready to use	1 x 3.5 ml
Wash Buffer, 10x concentrate	1 x 125 ml
Conjugate, ready to use	1 x 24 ml
TMB Substrate, ready to use	1 x 24 ml
Stop Solution, ready to use	1 x 24 ml
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Intended use

The flocktype AIV Ab is a specific and sensitive ELISA for detecting antibodies to Avian Influenza A Virus in serum and plasma samples from chicken and turkey.

The kit is approved by the Friedrich-Loeffler-Institute and licensed in accordance with § 11 (2) of the German Animal Health Act (FLI-B 435) for use in Germany for veterinary diagnostic procedures.

For veterinary use only.

Symbols

 Legal manufacturer

 Lot number

 Use by date

 Temperature limitations for storage

 Handbook

 Catalog number

 Material number

 For chicken and turkey samples

Quality control

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

Storage

The components of the flocktype AIV 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 from your local sales representative or by Email request under **compliance@indical.com**.

CAUTION: The Stop Solution contains 0.5 M sulfuric acid.

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

Introduction

The flocktype AIV Ab is a highly sensitive and specific solution for the detection of antibodies to Avian Influenza A Virus (AIV) in avian serum and plasma samples.

Avian influenza is caused by various strains of Influenza A Virus. It infects wild fowl as well as domestic poultry. Influenza A Virus strains are classified as low pathogenic or highly pathogenic. Highly pathogenic strains belong to subtypes H5 or H7 and can cause the severe systemic symptoms known as bird flu or avian flu. The flocktype AIV Ab uses a structural protein of AI virus prepared by recombinant technology as antigen. This protein is highly conserved amongst AIV strains and strongly immunogenic. Thus, all subtypes of Influenza A viruses will be detected.

Principle

The microtiter test plate is coated with a recombinant structural protein from the virus. During sample incubation AIV-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 15 minutes. In the presence of AIV-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-AIV 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
- Optional: Device for delivery and aspiration of Wash Buffer
- Microtiter plate absorbance reader
- Tubes or plates for diluting the samples
- Distilled water

Important notes

General precautions

The user should always pay attention to the following:

- Do not expose the TMB Substrate Solution to intense light or to sunlight when performing the test.
- Components of the test kit should not be contaminated.
- Do not use the components of the test kit past the expiration date.
- Water from ion-exchange systems used for diluting the Wash Buffer (10x) may interfere with the assay if not pure enough. Use double-distilled water or highly purified water (Milli-Q®).
- For accurate test results, it is essential to use clean glassware and to pipet and rinse carefully and strictly adhere to the incubation times when performing the test.

Protocol: ELISA test procedure

Important points before starting

- Please read „Important notes“ on page 8 before starting.

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.
- 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 samples: 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 microtitre 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 a dilution.

Protocol: ELISA

Please read „Things to do before starting“, page 9.

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.

   Note: 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 (1x) Wash Buffer. Remove the buffer after each rinse by aspiration or tapping.
5. Pipet 100 µl ready to use Conjugate to each well and incubate for 30 min at room temperature (18-25°C).
6. Remove solution from wells by aspiration or tapping.
7. Rinse each well 3x with 300 µl of prepared (1x) Wash Buffer. Remove the buffer after each rinse by aspiration or tapping.
8. Pipet 100 µl TMB Substrate Solution to each well.
9. Incubate for 15 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 carefully reading the instructions for use.

Calculation

Calculate the MV of the measured OD for the Negative Control (NC) and the Positive Control (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:

$$Log_{10}\ Titer = 1.54\ (Log_{10}\ S/P) + 3.77$$

Interpretation of the results

- Samples with the S/P ratio < 0.3 are negative. Specific antibodies to AIV could not be detected.
- Samples with the S/P ratio ≥ 0.3 are positive. Specific antibodies to AIV were detected.

INDICAL offers a range of ELISA kits and real-time PCR and real-time RT-PCR kits for the detection of animal pathogens.
Visit **www.indical.com** for more information about afosa, bactotype, cador, cattletype, flocktype, pigtype, Svanovir and virotype products.

For up-to-date licensing information and product-specific disclaimers, see the respective INDICAL kit handbook or user manual.

Limited License Agreement for flocktype AIV Ab

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Change index

Handbook	Version	Change
HB-1589-EN-004	December 2022	Editorial changes
HB-1589-EN-003	June 2022	Change TMB incubation time to 15 min
HB-1589-002	May 2018	INDICAL design

Quick guide for flocktype AIV Ab

Sample dilution:

Serum, plasma 1:500, mix well

Step	Protocol
1. Sample	100 µl/ well
2. Incubation	30 min at RT
3. Wash	3 x 300 µl
4. Conjugat	100 µl/ well
5. Incubation	30 min at RT
6. Wash	3 x 300 µl
7. TMB	100 µl/ well
8. Incubation	15 min at RT
9. Stop	100 µl/ well
10. Read	450 nm

Data interpretation

	Negative	Positive
Serum, plasma	S/P < 0.3	S/P ≥ 0.3

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Technical Support: **support@indical.com**
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