

flocktype[®]

Mycoplasma Mg Ab

Handbook

For detection of antibodies to *Mycoplasma gallisepticum* (Mg)

Licensed in accordance with § 11 (2) of the German Animal Health Act
MA No.: FLI-C 212



5 plates (cat. no. FT274703)



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Kit contents

flocktype Mycoplasma Mg Ab	(5)
Cat. no.	FT274703
Number of plates	5
Test Plate: microtiter plate with 96 wells, coated with non-infectious Mg antigen	5
Sample Diluent, ready to use	2 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	2 x 125 ml
Conjugate, ready to use	1 x 60 ml
TMB Substrate, ready to use	1 x 60 ml
Stop Solution, ready to use	1 x 60 ml
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Intended use

The flocktype Mycoplasma Mg Ab is a specific and sensitive indirect ELISA for the detection of antibodies to the *Mycoplasma gallisepticum* (Mg) in serum 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-C 212) 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 Mycoplasma Mg Ab is tested against predetermined specifications to ensure consistent product quality.

Storage

The components of the flocktype Mycoplasma Mg 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.

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 to 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

Mycoplasma gallisepticum (Mg) is a major avian pathogen and a primary agent of chronic respiratory disease in chickens and infectious sinusitis in turkeys. Lacking a cell wall, Mg is highly fragile in the environment but well adapted to colonize the respiratory tract, where it contributes to coughing, nasal discharge, reduced productivity, and increased susceptibility to secondary infections. Transmission occurs both horizontally through direct contact or aerosols, and vertically via contaminated eggs.

Mycoplasmas adhere to the respiratory epithelium and to erythrocytes. While cell-mediated immunity plays a key role in the host response to Mg, specific antibodies are also induced following both natural infection and vaccination. Because Mg infections may be subclinical and persist within flocks, robust serological monitoring is essential not only for early detection of infection but also for assessing vaccine responses and verifying the effectiveness of vaccination programs. Such surveillance supports flock health management and is integral to control and eradication strategies.

The flocktype Mycoplasma Mg Ab is designed to provide accurate detection of Mg-specific antibodies in chicken and turkey sera, supporting both infection surveillance and vaccine monitoring to enable timely interventions and improved disease control.

Principle

The microtiter plate is coated with non-infectious Mg antigens. During sample incubation Mg-specific antibodies bind to the immobilized antigen. Unbound material is removed by rinsing. The anti-IgY-horseradish peroxidase (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 Mg-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 value correlates with the concentration of anti-Mg 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.

- 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

- Spectrophotometer / 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 9 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 samples: Prior to sample analysis, dilute samples **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 samples can be diluted from a pre-dilution (1:50 in Sample Diluent, e.g. dilute 5 µl sample in 245 µl 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 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 diluted samples into the Test Plate wells.
- 1a. Alternatively, pipet 90 µl of Sample Diluent in each sample well and add 10 µl 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 (18-25°C) in the dark. Begin timing after the first well is filled.

10. Stop the reaction by adding 100 μ l 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 difference between the mean OD value for the Positive Control (PC) and the Negative Control (NC) must be ≥ 0.2 .
- The mean 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 mean value (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:

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

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

$$\text{Log}_{10} \text{ Titer} = 1.1 (\text{Log}_{10} S/P) + 3.5$$

Interpretation of the results

- **Samples with the S/P ratio < 0.3 or Titer < 841 are negative.**
Specific antibodies to *Mycoplasma gallisepticum* (Mg) were not detected.
- **Samples with the S/P ratio $\geq$ 0.3 or Titer $\geq$ 841 are positive.**
Specific antibodies to *Mycoplasma gallisepticum* (Mg) were detected.

INDICAL offers a comprehensive range of products to support animal pathogen testing including ELISA kits, qPCR kits and reagents, Master Mixes and nucleic acid extraction chemistry, and instrumentation for automated extraction.

Visit www.indical.com for more information about afosa, bactotype, cador, cattletype, flocktype, IndiField, IndiMag, IndiMix, IndiSpin, pigtype, SVANOVIR, and virotype products.

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Change index

Handbook	Version	Change
HB-2701-EN-001	April 2026	Product launch

Quick guide for flocktype Mycoplasma Mg Ab

Sample dilution

Serum: 1:500, mix well

Step	Protocol
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	15 min RT
9. Stop	100 µl/ well
10. Read	450 nm

Data interpretation

	Negative	Positive
Serum	S/P < 0.3 or Titer < 841	S/P ≥ 0.3 or Titer ≥ 841