

flocktype[®] Mycoplasma Mg Ab Handbook



2 (catalog no. 274502)



5 (catalog no. 274503)*

For the detection of antibodies to
Mycoplasma gallisepticum

REF

274502, 274503*



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* Available only on request.

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- Automation of sample and assay technologies

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Kit Contents











flocktype Mycoplasma Mg Ab		
Catalog no.	274502	274503*
Number of plates	2	5
Test Plate: microtiter plate with 96 wells, coated with non-infectious Mg 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 Mycoplasma Mg Ab* is a specific and sensitive ELISA for detecting antibodies to *Mycoplasma gallisepticum* in serum and plasma samples from chickens and turkeys. 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 and turkey samples

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. 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* Mycoplasma Mg Ab is tested against predetermined specifications to ensure consistent product quality.

Introduction

The flocktype Mycoplasma Mg Ab is a highly sensitive and specific solution for the detection of antibodies to *Mycoplasma gallisepticum* (*M. gallisepticum*) in serum and plasma samples from chickens and turkeys.

M. gallisepticum is found worldwide and causes chronic respiratory diseases, decreased growth, and reduced egg production in birds. Morbidity and mortality can vary widely and depend on stress conditions and secondary infections.

Mycoplasmas adhere to the respiratory epithelium and to erythrocytes. The cellular immune response is central to infections with *M. gallisepticum*, although the production of specific antibodies may also be induced by infections and vaccinations.

The flocktype Mycoplasma Mg Ab uses a membrane protein of *Mycoplasma gallisepticum* prepared by recombinant technology as antigen. This protein is highly conserved amongst the *Mycoplasma gallisepticum* strains and is strongly immunogenic.

The flocktype Mycoplasma Mg Ab in combination with the FlockSoft™ software is capable of calculating the antibody titer in chicken/turkey and of quantitatively depicting the results.

Principle

The microtiter plate is coated with a recombinant protein that is specific for *M. gallisepticum*. During sample incubation Mg-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 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 values correlate 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. 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 μ l sample in 499 μ l 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 μ l 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 μ l of Sample Diluent in each sample well and add 10 μ l 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 μ l of prepared Wash Buffer. Remove the buffer after each rinse.
5. Pipet 100 μ l 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 μ l of prepared Wash Buffer. Remove the buffer after each rinse.
8. Pipet 100 μ l 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 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 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:

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

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

$$\text{Log}_{10} \text{Titer} = 1.12 (\text{Log}_{10} S/P) + 3.83$$

Interpretation of the results

Samples with the S/P ratio <0.1 are negative.

Specific antibodies to *M. gallisepticum* could not be detected.

Samples with the S/P ratio ≥ 0.1 and <0.2 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.

Samples with the S/P ratio ≥ 0.2 are positive.

Specific antibodies to *M. gallisepticum* were detected.

For further diagnostic serological results, it is recommended to identify the pathogen obtained from tracheal swabs by real-time PCR (e.g., *bactotype*[®] Mycoplasma Mg/Ms PCR Kit).

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.
<i>flocktype</i> 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
<i>flocktype</i> Mycoplasma Mg Ab (5)*	For 480 reactions: 5 Test Plates, Wash Buffer, Sample Diluent, Positive Control, Negative Control, Conjugate, TMB Substrate Solution, Stop Solution	274503
Related products		
<i>flocktype</i> 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
<i>flocktype</i> 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
<i>bactotype</i> Mycoplasma Mg/Ms PCR Kit (96) [†]	For 96 reactions: Master Mix, Positive Control, Negative Control	288105

*Available only on request.

[†] Other kit sizes are available; see www.qiagen.com.

Product	Contents	Cat. no.
<i>flocktype</i> 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
<i>flocktype</i> 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
<i>flocktype</i> 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
<i>flocktype</i> 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
<i>flocktype</i> 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 < 0.1	S/P ≥ 0.1 and < 0.2	S/P ≥ 0.2

Notes

Notes

Trademarks: QIAGEN®, *bactotype*®, *cador*®, *cattletype*®, *flocktype*®, FlockSoft™, *pigtype*®, *virotypes*® (QIAGEN Group). Registered names, trademarks, etc. used in this document, even when not specifically marked as such, are not to be considered unprotected by law.

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