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bactotype® Mycoplasma Mg/Ms PCR Kit Handbook

For simultaneous detection of DNA from *Mycoplasma gallisepticum* and *Mycoplasma synoviae*

REF 24 reactions (cat. no. BT288103)

REF 96 reactions (cat. no. BT288105)

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Contents

Kit contents 3
Intended use 3
Symbols 4
Quality control 4
Storage 5
Safety information 5
Introduction 6
Principle 6
DNA extraction 7
Equipment and reagents to be supplied by user 9
Important notes 10
General precautions 10
Protocol: Real-time PCR for simultaneous detection of DNA from *Mycoplasma gallisepticum* and *Mycoplasma synoviae* 12
Important points before starting 12
Things to do before starting 12
Procedure 13
Data analysis and interpretation 15
Interpretation of results 15
Change index 19

Kit contents

bactotype Mycoplasma Mg/Ms PCR Kit **Cat. no.** **Number of reactions**	**(24)** **BT288103** **24**	**(96)** **BT288105** **96**
Master Mix (tube with orange cap) includes enzymes, primers, and probes	1x 500 µl	2 x 980 µl
Positive Control (tube with red cap)	1 x 25 µl	1 x 150 µl
Negative Control (tube with blue cap)	1 x 25 µl	1 x 150 µl
Handbook	1	1

Intended use

The bactotype Mycoplasma Mg/Ms PCR Kit is intended for the simultaneous detection of both *Mycoplasma gallisepticum* (Mg) and *Mycoplasma synoviae* (Ms) DNA from tracheal and oropharyngeal swabs of chicken and turkey and from culture medium.

For veterinary use only.

Symbols

 Legal manufacturer

 Lot number

 Use by date

 Temperature limitations for storage

 Handbook

 Catalog number

 Material number

 Protect from light

 For chicken and turkey samples

Quality control

In accordance with INDICAL's ISO-certified Quality Management System, each lot of bactotype Mycoplasma Mg/Ms PCR Kit is tested against predetermined specifications to ensure consistent product quality.

Storage

The components of the bactotype Mycoplasma Mg/Ms PCR Kit should be stored at -30°C to -15°C and are stable until the expiration date stated on the label. Avoid repeated thawing and freezing (>2x), as this may reduce assay sensitivity. Freeze the components in aliquots if they will only be used intermittently.

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**.

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

Introduction

The bactotype Mycoplasma Mg/Ms PCR Kit is a highly sensitive solution for the detection of DNA from *Mycoplasma gallisepticum* (Mg) and *Mycoplasma synoviae* (Ms) in samples from chicken and turkey. The multiplex PCR kit ensures the early and reliable detection of both pathogens in individual as well as in pooled samples from swabs (pool size up to 10 individual samples) and culture medium.

Mycoplasma infections are spread worldwide and cause severe economic losses in poultry farms due to chronic respiratory diseases (CRD), reduced growth rates, and loss of egg production. Morbidity and mortality can vary widely and depend on environmental conditions (e.g., stress) and secondary infections (other *Mycoplasma* species, bacteria or viruses). *Mycoplasma gallisepticum* can cause chronic respiratory diseases in chicken and sinusitis in turkeys. Infection with *Mycoplasma synoviae* leads to subclinical disease of the upper respiratory tract and even to synovitis, tendovaginitis and bursitis.

Principle

Polymerase chain reaction (PCR) is based on the amplification of specific regions of the pathogen genome. In real-time PCR, the amplified product is detected using fluorescent dyes. These are usually linked to oligonucleotide probes that bind specifically to the amplified product. Monitoring the fluorescence intensities during the PCR run (i.e., in real time) allows the detection of the accumulating product without the need to re-open the reaction tubes afterward.

The bactotype Mycoplasma Mg/Ms PCR Kit contains all of the necessary reagents for the detection of Mg and Ms DNA, including a positive and negative control.

An internal control excludes the possibility of false-negative results.

The kit uses three specific primer/probe combinations:

- Cy®5 fluorescence for DNA from *Mycoplasma gallisepticum*
- FAM™ fluorescence for DNA from *Mycoplasma synoviae*
- HEX™ fluorescense for the Internal Control (β-actin DNA, present within the sample)

A Positive Control serves to verify the functionality of the pathogen assay, for example, the correct setup of the reaction mix.

DNA extraction

The bactotype Mycoplasma Mg/Ms PCR Kit is intended for the simultaneous detection of both *Mycoplasma gallisepticum* and *Mycoplasma synoviae* DNA from tracheal and oropharyngeal swabs of chicken and turkey and from culture medium.

Due to the high sensitivity of the test, pools of up to 10 individual swab samples can be tested.

Prior to real-time PCR, DNA must be extracted from the starting material. INDICAL offers a range of validated kits for the extraction of DNA from animal samples.

- QIAamp® cador® Pathogen Mini Kit
- virotype® Tissue Lysis Reagent

If real-time PCR is not performed immediately after extraction, store the DNA at -20°C or at -70°C for longer storage.

For rapid preparation of swab samples, without DNA extraction, INDICAL recommends virotype Tissue Lysis Reagent. Lysates from swabs should be stored at -20°C or at 2-8°C for up to 5 days.

For further information on automated and manual extraction of Mg or Ms DNA from different sample types, refer to the respective handbook or contact INDICAL Support at **support@indical.com**.

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.

- Pipets
- Nuclease-free, aerosol-resistant pipet tips with filters
- Sterile 1.5 ml Eppendorf® tubes
- Nuclease-free (RNase/DNase-free) consumables. Special care should be taken to avoid nuclease contamination of all reagents and consumables used to set up PCR for sensitive identification of viral nucleic acids
- Cooling device or ice
- Benchtop centrifuge with rotor for 1.5 ml tubes
- Real-time cycler with appropriate fluorescent channels
- Appropriate software for chosen real-time cycler
- Appropriate strip tubes and caps or 96-well optical microplate with optical sealing film or cover for chosen real-time cycler

Important notes

General precautions

The user should always pay attention to the following:

- Use nuclease-free pipet tips with filters.
- Store and extract positive materials (specimens, positive controls and amplicons) separately from all other reagents, and add them to the reaction mix in a spatially separated facility.
- Thaw all components on ice before starting as assay.
- When thawed, mix the components by inverting and centrifuge briefly.
- Do not use components of the test kit past the expiration date.
- Keep samples and controls on ice or in a cooling block during the setup of reactions.

Negative control

At least one negative control reaction should be included in each PCR run. This enables assessment of contamination in the reaction.

Positive control

When performing PCR on unknown samples, it is recommended to perform a positive control reaction in the PCR run, containing a sample that is known to include the targeted DNA. A positive control serves to prove the functionality of the pathogen assay, for example, the correct setup of the reaction mix. Use 5 µl of the Positive Control provided with

the bactotype Mycoplasma Mg/Ms PCR Kit to test for successful amplification of the target.

Extraction and amplification control

For increased process safety and convenience, an extraction and amplification control assay is included in the form of a primer/probe set that detects a housekeeping gene present within the sample. This allows both extraction and amplification to be monitored.

Protocol: Real-time PCR for simultaneous detection of DNA from *Mycoplasma gallisepticum* and *Mycoplasma synoviae*

Important points before starting

- Please read „Important notes“ on page 10 before starting.
- Include at least one positive control (Positive Control) and one negative control (Negative Control) per PCR run.
- Before beginning the procedure, read through the protocol and ensure that you are familiar with the operation of the chosen real-time PCR cycler.
- Perform the protocol without interruption.

Things to do before starting

- Thaw all reagents on ice and protect from light.
- Maintain reagents on ice during PCR setup.
- Before use, spin the reagents briefly.

Procedure

1. Pipet 20 µl of the Master Mix into each reaction tube. Then add 5 µl of the sample DNA (Table 1).

   Include positive and negative control reactions.

   Positive Control: Use 5 µl of the positive control (Positive Control) instead of sample DNA.

   Negative Control: Use 5 µl of the negative control (Negative Control) instead of sample DNA.

Table 1. Preparation of reaction mix

Component	**Volume**
Master Mix	20 µl
Sample	5 µl
Total volume	**25 µl**

2. Close the reaction tubes with the corresponding caps.
3. Set the filters for the reporter dyes in the software of your thermal cycler according to Table 2.

Table 2. Filter settings for the reporter

Pathogen/ Internal Control	**Reporter**
Mycoplasma gallisepticum	Cy5
Mycoplasma synoviae	FAM
Internal Control	HEX/ JOE™[1]
Passive reference[2]	ROX™

1 Use the option appropriate for your thermal cycler.
2 Internal reference for use on ABI PRISM® Sequence Detection Systems (Applied Biosystems®)

4. Run the real-time PCR protocol according to Table 3 if running only the bactotype Mycoplasma Mg/Ms PCR Kit.

Table 3. Real-time PCR protocol for bactotype Mycoplasma Mg/Ms PCR Kit

Step	Temperature	Time	Number of cycles
Initial Activation	95°C	5 min	1
3-step cycling			
Denaturation	95°C	15 s	40
Annealing*	60°C	30 s	
Extension	68°C	30 s	

* Fluorescence data collection.

5. Run the real-time RT-PCR protocol according to Table 4 if running the bactotype Mycoplasma Mg/Ms PCR it simultaneously with the virotype Influenza A RT-PCR Kit.

Table 4. virotype Influenza A RT-PCR protocol for simultaneous assays

Step	Temperature	Time	Number of cycles
Reverse Transcription	45°C	10 min	1
Initial Activation	95°C	10 min	1
2-step cycling			
Denaturation	95°C	15 s	40
Annealing/ Extension*	60°C	60 s	

* Fluorescence data collection.

Data analysis and interpretation

Interpretation of results

For the assay to be valid the Positive Control must give a signal in the Cy5, FAM and HEX channels with a C_T[1] < 35. The Negative Control must not give a fluorescence signal.

The following results are possible if working with unknown samples. The possible sample results are also summarized in Table 5 on page 18.

The sample is positive for Mg, and the assay is valid, if the following criteria are met:

- The sample yields a signal in both the Cy5 and the HEX channel.
- The Positive Control yields a signal in the Cy5, FAM and HEX channel.
- The Negative Control does not yield a signal in any of the channels.

Note that very high concentrations of Mg DNA in the sample may lead to a reduced HEX signal or no HEX signal due to competition with the internal control.

The sample is positive for Ms, and the assay is valid, if the following criteria are met:

- The sample yields a signal in both the FAM and the HEX channel.
- The Positive Control yields a signal in the Cy5, FAM and HEX channel.

[1] Threshold cycle (CT) — cycle at which the amplification plot crosses the threshold, i.e., there is the first clearly detectable increase in fluorescence

- The Negative Control does not yield a signal in any of the channels.

Note that very high concentrations of Ms DNA in the sample may lead to a reduced HEX signal or no HEX signal due to competition with the internal control.

The sample is positive for Mg and Ms, and the assay is valid, if the following criteria are met:

- The sample yields a signal in the Cy5, FAM and HEX channel.
- The Positive Control yields a signal in the Cy5, FAM and HEX channel.
- The Negative Control does not yield a signal in any of the channels.

Note that very high concentrations of *Mycoplasma* DNA in the sample may lead to a reduced HEX signal or no HEX signal due to competition with the internal control.

The sample is negative for Mg and Ms, and the assay is valid, if the following criteria are met:

- The sample yields a signal only in the HEX channel.
- The Positive Control yields a signal in the Cy5, FAM and HEX channel.
- The Negative Control does not yield a signal in any of the channels.

A positive HEX signal means that extraction and amplification were successful as the housekeeping gene within the sample is amplified.

Analysis of swab material

The sample results are inconclusive, and the assay is invalid, if the following occurs:

- The sample yields no signal in any of the channels.

The PCR was inhibited or the sample extraction was incorrect. It is recommended to retest the respective individual samples in nuclease free water (e.g., diluted 1:5), to repeat the DNA extraction, or repeat the whole test procedure starting with new sample material.

Analysis of cultured material

The sample contains no Mg or Ms DNA if the following occurs:

- The sample yields no signal in any of the channels.

However, due to the lack of poultry-specific β-actin DNA in the cultured material, no information about PCR inhibition or incorrect extraction is given.

Check that there is a fluorescence signal in the all channels for the positive control reaction (Positive Control). Absence of a signal for the Positive Control indicates an error, which could be due to DNA extraction failure, incorrect setup of the master mix, or incorrect cycling conditions.

Repeat DNA extraction or repeat the whole procedure starting with new sample material.

Table 5. Results interpretation table*

Cy5	FAM (Pathogen)	HEX	Sample result Positive for:
X		(X)	*M. gallisepticum*
	X	(X)	*M. synoviae*
X	X	X	*M. gallisepticum* and *M. synoviae*
		X	negative
			inconclusive

* Interpretation of sample results can be determined provided positive and negative control reactions are performed. The Positive Control must yield a signal in the Cy5, FAM and HEX channel. The Negative Control must yield no signal in any of the channels. For a complete explanation of possible sample results please refer to “Data analysis and interpretation” on page 15.

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 bactotype, cador, cattletype, flocktype, pigtype and virotype products.

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

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Change index

Handbook	Version	Change
HB-1601-EN-004	August 2018	INDICAL design

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