November 2014

bactotype® Mycoplasma Mg/Ms PCR Kit Handbook

24 (catalog no. 288103)

96 (catalog no. 288105)

480 (catalog no. 288107)*

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

REF 288103, 288105, 288107*

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QIAGEN®

* Available only on request.

Sample & Assay Technologies

QIAGEN Sample and Assay Technologies

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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

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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

***bactotype* Mycoplasma Mg/Ms PCR Kit**	**(24)**	**(96)**	**(480)**
Catalog no.	**288103**	**288105**	**288107***
Number of reactions	**24**	**96**	**480**
Master Mix (tube with orange cap) includes enzymes, primers and probes	1 x 500 µl	2 x 980 µl	6 x 1625 µl
Positive Control (tube with red cap)	1 x 25 µl	1 x 70 µl	2 x 50 µl
Negative Control (tube with blue cap)	1 x 25 µl	1 x 70 µl	2 x 50 µl
Handbook	1	1	1

* Available only on request.

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

Contains reagents for <N> tests


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 *bactotype* Mycoplasma Mg/Ms PCR Kit should be stored at –15 to –30°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 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.

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

Quality Control

In accordance with QIAGEN'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.

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.
The kit uses three specific primer/probe combinations: one for Mg yielding Cy5™ fluorescence, one for Ms yielding FAM™ and one for a housekeeping gene (β-actin), present within the sample, yielding HEX™ fluorescence.

The Positive Control contains Mg/Ms DNA and serves to prove 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* (Mg) and *Mycoplasma synoviae* (Ms) 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. QIAGEN offers a range of products for DNA extraction from animal samples.

- QIAamp® *cador*® Pathogen Mini Kit
- QIAamp DNA Mini Kit
- DNeasy® Blood and Tissue Mini Kit
- *virotype*® Tissue Lysis Reagent

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

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

DNA extraction using kits based on spin-column technology can be automated using the QIAcube®.

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 nucleic acids.
- Cooling device or ice
- Benchtop centrifuge with rotor for 1.5 ml tubes

- Rotor-Gene® Q or 96-well plate real-time cycler with appropriate fluorescent channels
- Rotor-Gene Q software version 1.7.94 or higher, or appropriate software for chosen 96-well plate cycler
- Strip Tubes and Caps, 0.1 ml, for use with Rotor-Gene Q (cat. no. 981103 or 981106) or 96-well optical microplate with optical sealing film or cover for chosen 96-well plate 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 an assay.
- When thawed, mix the components by inverting and centrifuge briefly.
- Do not use components of the test reagent 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 identification of *Mycoplasma gallisepticum* and *Mycoplasma synoviae*

Important points before starting

- Please read "Important Notes" on page 11 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. Select the red, green, and yellow channels on the Rotor-Gene Q.

Table 2. Filter settings for reporter

Component	Reporter
Mg	Cy5
Ms	FAM
Internal control	HEX/JOE®*
Passive reference†	ROX™

* Use the option appropriate for your thermal cycler.

† Internal reference for use with the Applied Biosystems® ABI PRISM® Sequence Detection Systems.

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 Mycoplasma Mg/Ms

Temperature	Time	Number of cycles
95°C	5 min	1
95°C	15s	40
60°C*	30 s	
68°C	30 s	

* Fluorescence data collection.

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

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

Temperature	Time	Number of cycles
45°C	10 min	1
95°C	10 min	1
95°C	15 s	40
60°C†	60 s	

† Fluorescence data collection.

Data Analysis and Interpretation

Interpretation of results

For the assay to be valid the Cy5, FAM, and HEX fluorescence of the Positive Control must give a signal with a C_T* value of <35. The Negative Control must give no signal.

The following results are possible if working with unknown samples. The possible sample results are also summarised in Table 5 on page 19.

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 HEX† channels
- The Positive Control yields a signal in the Cy5, FAM, and HEX
- The Negative Control yields no signal

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.

* Threshold cycle (C_T) — cycle at which the amplification plot crosses the threshold, i.e., there is the first clearly detectable increase in fluorescence.
† Red and yellow on the Rotor-Gene Q.

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 HEX* channel
- The Positive Control yields a signal in the Cy5, FAM, and HEX
- The Negative Control yields no signal

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 both 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
- The Negative Control yields no signal

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.

* Green and yellow on the Rotor-Gene Q.

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

- The sample yields a signal in only HEX channel
- The Positive Control yields a signal in the Cy5, FAM, and HEX
- The Negative Control yields no signal

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:

- No fluorescence signal is detected

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:

- No fluorescence signal is detected

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*

	Pathogen genotype				
Fluorescence signal	**Mg**	**Ms**	**Mg + Ms**	**Negative**	**Invalid**
Cy5	X		X		
FAM		X	X		
HEX	(X)	(X)	(X)	X	

* 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 channels. 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 16.

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.
bactotype Mycoplasma Mg/Ms PCR Kit (24)	For 24 reactions: Master Mix, Positive Control, Negative Control	288103
bactotype Mycoplasma Mg/Ms PCR Kit (96)	For 96 reactions: Master Mix, Positive Control, Negative Control	288105
bactotype Mycoplasma Mg/Ms PCR Kit (480)*	For 480 reactions: Master Mix, Positive Control, Negative Control	288107
Related products		
bactotype MAP PCR Kit (24)†	For 24 reactions: Master Mix, Internal Control DNA, Positive Control, Negative Control	285903
flocktype Mycoplasma Mg Ab (5)†	For 480 reactions: 5 Test Plates (strips), Wash Buffer, Sample Diluent, Positive Control, Negative Control, Anti-IgY-HRP Conjugate, TMB Substrate Solution, Stop Solution	274503

* Available only on request.

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

Product	Contents	Cat. no.
flocktype Mycoplasma Ms Ab (5)*	For 480 reactions: 5 Test Plates (strips), Wash Buffer, Sample Diluent, Positive Control, Negative Control, Anti-IgY-HRP Conjugate, TMB Substrate Solution, Stop Solution	274603
flocktype Mycoplasma Mg/Ms Ab (5)*	For 480 reactions: 5 Test Plates, Wash Buffer, Sample Diluent, Positive Control, Negative Control, Anti-IgY-HRP Conjugate, TMB Substrate Solution, Stop Solution	274803
virotype ASFV PCR Kit (96)	For 96 reactions: Master Mix, Positive Control, Negative Control	281905
virotype Influenza A RT-PCR Kit (96) *	For 96 reactions: Master Mix, Positive Control, Negative Control	282605
virotype BTV RT-PCR Kit (96)*	For 96 reactions: PCR Mix, Enzyme Mix, Positive Control, Negative Control	280435
virotype BTV pan/8 RT-PCR Kit (96)*	For 96 reactions: Master Mix, Positive Control, Negative Control	280445

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

Product	Contents	Cat. no.
virotype BVDV RT-PCR Kit (96)*	For 96 reactions: PCR Mix, Enzyme Mix, Positive Control, Negative Control	280375
virotype PRRSV RT-PCR Kit (96)*	For 96 reactions: Master Mix, Positive Control, Negative Control	282305
virotype CSFV RT-PCR Kit (96)*	For 96 reactions: Master Mix, Positive Control, Negative Control	281805
virotype SBV RT-PCR Kit (96)*	For 96 reactions: Master Mix, Positive Control, Negative Control	281605
QIAamp *cador* Pathogen Mini Kit (50)*	For 50 preps: 50 QIAamp Mini Spin Columns, Carrier RNA, Proteinase K, Collection Tubes (2 ml), RNase-free Buffers	54104
QIAamp DNA Mini Kit (50)*	For 50 DNA preps: 50 QIAamp Mini Spin Columns, QIAGEN Proteinase K, Reagents, Buffers, Collection Tubes (2 ml)	51304
DNeasy Blood & Tissue Kit (50)*	For 50 preps: 50 DNeasy Mini Spin Columns, Proteinase K, Buffers, Collection Tubes (2 ml)	69504

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

Product	Contents	Cat. no.
virotype TLR (100 ml)*	100 ml Tissue Lysis Reagent	289992
Rotor-Gene Q 5plex Platform	Real-time PCR cycler with 5 channels (green, yellow, orange, red, crimson), laptop computer, software, accessories, 1-year warranty on parts and labor	9001570

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* products.

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.

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

Notes

Notes

Limited License Agreement for *bactotype* Mycoplasma Mg/Ms PCR Kit

Use of this product signifies the agreement of any purchaser or user of the product to the following terms:

1. The product may be used solely in accordance with the protocols provided with the product and this handbook and for use with components contained in the kit only. QIAGEN grants no license under any of its intellectual property to use or incorporate the enclosed components of this kit with any components not included within this kit except as described in the protocols provided with the product, this handbook, and additional protocols available at www.qiagen.com. Some of these additional protocols have been provided by QIAGEN users for QIAGEN users. These protocols have not been thoroughly tested or optimized by QIAGEN. QIAGEN neither guarantees them nor warrants that they do not infringe the rights of third-parties.

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