April 2015

bactotype® MAP PCR Kit Handbook

24 (catalog no. 285903)

96 (catalog no. 285905)

480 (catalog no. 285907)*

For detection of DNA from *Mycobacterium avium* subsp. *paratuberculosis*

Licensed in accordance with §11 (2) of the German Animal Health Act (FLI-B 651)

REF 285903, 285905, 285907*

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* Available only on request.

Sample & Assay Technologies

QIAGEN Sample and Assay Technologies

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- 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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Contents

Kit Contents

bactotype MAP PCR Kit	(24)	(96)	(480)*
Catalog no.	**285903**	**285905**	**285907**
Number of reactions	**24**	**96**	**480**
Master Mix (tube with orange cap) includes enzymes, primers, and probes	1 x 425 µl	2 x 840 µl	6 x 1390 µl
Internal Control DNA	1 x 30 µl	1 x 110 µl	1 x 550 µl
Positive Control (tube with red cap)	1 x 50 µl	1 x 120 µl	2 x 240 µl
Negative Control (tube with blue cap)	1 x 50 µl	1 x 120 µl	2 x 240 µl
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* Available only on request.

Intended Use

The *bactotype* MAP PCR Kit is intended for the detection of DNA from *Mycobacterium avium* subsp. *paratuberculosis* (MAP) in fecal and tissue samples (e.g., small intestine, mesenterial lymph nodes) from ruminants and culture slants (individual and pooled samples). Up to 5 individual samples can be tested in a pool. The kit is approved by the Friedrich-Loeffler-Institut and licensed in accordance with § 11 (2) of the German Animal Health Act (FLI-B 651) for use in Germany for veterinary diagnostic procedures. 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 ruminant samples

Storage

The components of the *bactotype* MAP 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* MAP PCR Kit is tested against predetermined specifications to ensure consistent product quality.

Introduction

The *bactotype* MAP PCR Kit is a highly sensitive and specific solution for the detection of DNA from *Mycobacterium avium* subsp. *paratuberculosis* (MAP) in samples from ruminants. MAP is the cause of paratuberculosis (Johne´s disease), a chronic inflammatory intestinal disease of ruminants, which occurs worldwide. Domestic and wild ruminants as well as camelids can be infected. Clinical signs such as chronic diarrhea, edema, and progressive weight loss develop at the late stage of the disease after several years of incubation. In small ruminants weight loss is more common than diarrhea. Paratuberculosis is an incurable and fatal disease.

The high sensitivity of *bactotype* MAP PCR Kit allows the detection of the pathogen in fresh, as well as cultured samples.

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 afterwards.

The *bactotype* MAP PCR Kit contains all of the necessary reagents for the detection of MAP DNA, including a positive control, a negative control, and an internal control.

The internal control DNA permits tests for successful purification and amplification by adding to the DNA purification procedure.

The kit uses two specific primer/probe combinations: one for MAP DNA yielding FAM™ fluorescence and one for the internal control yielding MAX™ fluorescence.

The Positive Control contains MAP DNA and serves to prove the functionality of the pathogen assay, for example, the correct setup of the reaction mix.

DNA extraction

The *bactotype* MAP PCR Kit can be used for the detection of MAP DNA in fecal and tissue samples (e.g., small intestine, mesenterial lymph nodes) from ruminants and culture slants. Up to 5 individual samples can be tested in a pool.
Prior to real-time PCR, DNA must be extracted from the starting material. Internal Control DNA must be added to the lysis buffer prior to extraction procedure. In most cases 1 µl Internal Control DNA per sample is suitable. For additional information please refer to the Supplementary protocols.

Fecal sample handling recommendations:
Transport fecal samples at 4°C, or at –15 to –30°C.

Preparation of fecal samples

Before MAP DNA is extracted from fecal samples the starting material must be thoroughly mixed. Often times the pathogen is uneven distributed in the samples.

Afterwards the sample is homogenized in the lysis buffer. In order to ensure correct homogenization, an appropriate lysis matrix (beads) as well as suitable instrumentation should be used.

Additional supplementary protocols for the extraction of MAP DNA from fecal samples using the following kits can be found at:

- QIAamp® *cador*® Pathogen Mini Kit
  (go to "Resources" at: www.qiagen.com/cadorpathogen)
- QIAamp DNA Stool Mini Kit
  (go to "Resources" at: www.qiagen.com/DNAstool)

Alternatively, further available extraction methods, which have been validated for the treatment of MAP positive fecal samples, can be used (e.g. containing additional concentration steps).

Preparation of tissue samples

Please refer to Supplementary Protocol for Purification of MAP DNA from tissue using one of the following kits:

- QIAamp® *cador*® Pathogen Mini Kit
  (go to "Resources" at: www.qiagen.com/cadorpathogen)

Preparation of culture slants

Please refer to the protocols provided in the kit handbooks, including pretreatment steps, for the purification of MAP DNA from culture slants (in PBS or 0.9% sodium chloride solution), using one of the following kits:

- QIAamp *cador* Pathogen Mini Kit
  (Pretreatment B2 for Difficult-to-Lyse Bacteria in Cell-Free Fluids)

- DNeasy Blood & Tissue Kit
  (Pretreatment for Gram-Positive Bacteria)

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

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.
- Benchtop centrifuge with rotor for 1.5 ml tubes
- Cooling device or ice
- 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 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 bacterial DNA. A positive control serves to prove the functionality of the pathogen assay, for example, the correct setup of the reaction mix. Use 8 µl of the Positive Control provided with the *bactotype* MAP 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 an Internal Control DNA. It is strongly recommended to add the Internal Control DNA to the sample lysis solution to monitor the extraction and amplification.

Protocol: Real-time PCR for detection of DNA of *Mycobacterium avium* subsp. *paratuberculosis*

Important points before starting

- Please read "Important Notes" on page 12 before starting.
- Internal Control DNA is supplied. This allows the user to control the DNA isolation procedure and to check for possible PCR inhibition. The internal control should be added directly to the lysis.
- 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 17 µl of the Master Mix into each reaction tube. Then add 8 µl of the extracted sample DNA (Table 1).**
   Include positive and negative control reactions.
   Positive control: Use 8 µl of the positive control (Positive Control) instead of sample DNA.
   Negative control: Use 8 µl of the negative control (Negative Control) instead of sample DNA.

Table 1. Preparation of reaction mix

Component	Volume
Master Mix	17 µl
Sample	8 µl
Total volume	25 µl

2. **Close the reaction tubes with the corresponding caps.**
3. **Set the filters for the reporter and quencher dyes in the software of your thermal cycler according to Table 2. Select the green and yellow channels on the Rotor-Gene Q.**

   Important: Set a fixed gain of +4 in the green and +1 in the yellow channel to ensure optimal fluorescence gains for the MAP and the Internal Control Assays.

Table 2. Filter settings for reporter

Pathogen/internal control	Reporter	Rotor-Gene Q
MAP	FAM	Green
Internal control	MAX*/HEX™/JOE™†	Yellow
Passive reference‡	ROX™	

* MAX NHS Ester as reporter dye has an excitation/emission maxima of 524/557 nm, allowing detection in the same channel as HEX or JOE and therefore can be used with most real-time cyclers.

† 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.**

Table 3. Real-time PCR protocol

Temperature	Time	Number of cycles
95°C	15 min	1
95°C	15 s	40
60°C*	30 s	
72°C	35 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 both the FAM and MAX/ HEX channel with a C_T*<35. The Negative Control must not give a signal in the FAM and MAX/ HEX channel. The following results are possible if working with unknown samples. The possible sample results are also summarized in Table 4 on page 19.

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

- The sample yields a signal in both the FAM and MAX/ HEX† channel.
- The Positive Control yields a signal in both the FAM and MAX/ HEX channel.
- The Negative Control does not yield a signal in the FAM and MAX/ HEX channel.

Note that very high concentrations of MAP DNA in the sample may lead to a reduced MAX/ HEX signal or no MAX/ 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.

† Green and yellow on the Rotor-Gene Q.

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

- The sample yields a signal in the MAX/ HEX channel but not in the FAM channel.
- The Positive Control yields a signal in both the FAM and MAX/ HEX channel.
- The Negative Control does not yield a signal in the FAM and MAX/ HEX channel.

A positive MAX/ HEX signal means that extraction and amplification were successful.

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

- The sample yields no signal in the FAM and MAX/ HEX channel.

If no signal is detected in both the FAM (pathogen) and the MAX/ HEX (Internal Control, IC) channel, the result is inconclusive. The absence of a signal for the internal control indicates PCR inhibition and/or other malfunctions.

To check for inhibition, we recommend 1:5 dilution of the sample DNA in nuclease free water, to repeat the DNA extraction, or repeat the whole test procedure starting with new sample material.

Check that there is a fluorescence signal in the FAM channel for the positive control reaction (Positive Control). Absence of a signal for the Positive Control indicates an error, which could be

due to incorrect setup of the reaction mix or incorrect cycling conditions.

Table 4. Results interpretation table*

Sample result	Reporter	
	FAM (pathogen)	MAX/ HEX (IC)
MAP positive	X	X
MAP positive (strong positive)	X	
MAP negative		X
Inconclusive result		

* Interpretation of sample results can be determined provided positive and negative control reactions are performed. The Positive Control must yield a signal in both the FAM and MAX/ HEX channel. The Negative Control must yield no signal in the FAM and MAX/ HEX channel. For a complete explanation of possible sample results please refer to "Data Analysis and Interpretation" on page 17.

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 MAP PCR Kit (24)	For 24 reactions: Master Mix, Internal Control DNA, Positive Control, Negative Control	285903
bactotype MAP PCR Kit (96)	For 96 reactions: Master Mix, Internal Control DNA, Positive Control, Negative Control	285905
bactotype MAP PCR Kit (480)*	For 480 reactions: Master Mix, Internal Control DNA, Positive Control, Negative Control	285907
Related products		
cattletype MAP Ab (5)†	For 480 reactions: 5 Test Plates (strips), Wash Buffer, Sample Diluent, Positive Control, Negative Control, Conjugate, TMB Substrate Solution, Stop Solution	270803
bactotype Mycoplasma Mg/Ms PCR Kit (96)†	For 96 reactions: Master Mix, Positive Control, Negative Control	288105
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

* Available only on request.
† Other kit sizes are 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 ASFV PCR Kit (96)	Für 96 Reaktionen: Master-Mix, Positivkontrolle, Negativkontrolle	281905
virotype CSFV RT-PCR Kit (96)*	Für 96 Reaktionen: Master-Mix, Positivkontrolle, Negativkontrolle	281805
virotype SBV RT-PCR Kit (96)*	For 96 reactions: Master Mix, Positive Control, Negative Control	281605
virotype PRRSV RT-PCR Kit (96)*	For 96 reactions: Master Mix, Positive Control, Negative Control	282305
virotype Influenza A RT-PCR Kit (96)*	For 96 reactions: Master Mix, Positive Control, Negative Control	282605
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

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

Product	Contents	Cat. no.
QIAamp DNA Stool Mini Kit (50)	For 50 RNA preps: 50 QIAamp Mini Spin Columns, carrier RNA, Collection Tubes (2 ml), RNase-Free buffers	51504
DNeasy Blood & Tissue Kit (50)*	For 50 DNA preps: 50 DNeasy Mini Spin Columns, Proteinase K, Buffers, Collection Tubes (2 ml)	69504

* 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*® 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.

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Limited License Agreement for *bactotype* MAP 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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3. This kit and its components are licensed for one-time use and may not be reused, refurbished, or resold.

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