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bactotype® MAP PCR Kit
Handbook

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

Licensed in accordance with § 11 (2) of the German Animal Health Act
MA No.: FLI-B 651

REF 96 reactions (cat. no. BT285905)

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INDICAL BIOSCIENCE

Contents

Kit contents

bactotype MAP PCR Kit	(96)
Cat. no.	BT285905
Number of reactions	96
Master Mix (tube with orange cap), includes enzymes, primers, and probes	2 x 840 µl
Internal Control DNA (tube with transparent cap)	1 x 110 µl
Positive Control (tube with red cap)	1 x 170 µl
Negative Control (tube with blue cap)	1 x 170 µl
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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

	Legal manufacturer
	Lot number
	Use by date
	Temperature limitations for storage
	Handbook
	Catalog number
	Material number
	Protect from light
	For ruminant samples

Quality control

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

Storage

The components of the bactotype MAP 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 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:

- FAM™ fluorescence for DNA from MAP
- MAX™ fluorescence to detect the internal control

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.

Fecal sample handling recommendation:
transport fecal samples at 4°C or -30°C to -15°C.

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 specific for each sample type.

INDICAL offers a range of validated kits for the extraction of DNA from animal samples.

Extraction based on magnetic beads:

- IndiMag® Pathogen Kit (SP947457)
- IndiMag Pathogen Kit w/o plastics (SP947257)
- IndiMag Pathogen IM2 Cartridge (SP957654C608)
- IndiMag Pathogen IM48 Cartridge (SP947654P608, SP947654P224)
- IndiMag Pathogen KF96 Cartridge (SP947855P196)

Extraction based on spin columns:

- IndiSpin® Pathogen Kit (SP54104, SP54106)
- IndiSpin QIAcube® HT Pathogen Kit (SP54161)

Note: depending on the sample type, specific pretreatments are necessary.

Pretreatment 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 are available from your local sales representative or by Email request under support@indical.com.

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).

Pretreatment of tissue samples

Additional supplementary protocols for the extraction of MAP DNA from fecal samples are available from your local sales representative or by Email request under support@indical.com.

Pretreatment of culture slants

Extraction of MAP DNA from culture slants (in PBS or 0.9% sodium chloride solution) can be done using one of the recommended kits. Note: use Pretreatment B2 for Difficult-to-Lyse Bacteria in Cell-Free Fluids

If real-time PCR is not performed immediately after extraction, store the DNA at -20°C or, for long-term storage, at -80°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 viral nucleic acids
- Instruments and consumables for homogenizing and mechanical disruption of samples
- 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, containing all the components of the reaction except for the pathogen template. 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, e.g., 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 11 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 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 dyes in the software of your thermal cycler according to Table 2.

Table 2. Filter settings for the reporter

Pathogen/ Internal Control	Reporter
MAP	FAM
Internal Control	MAX[1]/ HEX/ JOE[2]
Passive reference[3]	ROX

1 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.

2 Use the option appropriate for your thermal cycler.

3 Internal reference for use with the Applied Biosystems® 7500.

Note: When using Rotor-Gene® Q 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.

4. Run the real-time PCR protocol according to Table 3.

Table 3. Real-time PCR protocol for bactotype MAP

Step	Temperature	Time	Number of cycles
Initial activation	95°C	15 min	1
3-step cycling			
Denaturation	95°C	15 s	
Annealing*	60°C	30 s	40
Extension	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[1] < 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 18.

Note for users of ABI 7500 Real-Time PCR Systems: ABI 7500 users should note that solely relying on the automatic threshold setting (Auto CT) and Auto baseline setting could lead to a misinterpretation of results by the software. Results should always be verified and adjusted by manually setting the threshold, and deselect „Auto CT" and „Auto Baseline" setting respectively, where applicable. Refer to your instrument guideline for more information on how to perform the appropriate analysis settings.

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.

[1] Threshold cycle (C_T) — 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 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.

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[1]

Sample result	FAM (MAP)	MAX/ HEX (IC)
MAP positive	X	X
MAP positive (strong positive)	X	
MAP negative		X
inconclusive		

1 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 16.

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-1746-EN-007	Feb 2025	Discontinuation 24 & 480 rxn; Implementation new extraction kits
HB-1746-006	June 2018	INDICAL design; adjustments to chapter „DNA extraction“

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