May 2019 | EN

bactotype® C. burnetii PCR Kit Handbook

For the detection of DNA from *Coxiella burnetii*

Licensed in accordance with § 11 (2) of the German Animal Health Act
MA No.: FLI-C 071

96 reactions (cat. no. BT285885)

INDICAL BIOSCIENCE GmbH, Deutscher Platz 5b,
04103 Leipzig, Germany

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 detection of DNA from *Coxiella burnetii* ... 12
    Important points before starting ... 12
    Things to do before starting ... 13
    Procedure ... 13
Data analysis and interpretation ... 15
    Interpretation of results (qualitative analysis) ... 15
    Interpretation of results (quantitative analysis) ... 17
Change index ... 20

Kit contents

bactotype C. burnetii PCR Kit	**(96)**
Cat. no.	**BT285885**
Number of reactions	**96**
Master Mix (tube with orange cap), includes primers, probes and enzymes	2 x 980 µl
Positive Control (tube with red cap)	1 x 150 µl
Negative Control (tube with blue cap)	1 x 150 µl
Cox Standard (tube with transparent cap)	1 x 50µl
Handbook	1

Intended use

The bactotype C. burnetii PCR Kit is intended for the detection and quantification of DNA from *Coxiella burnetii* in milk (quarter milk samples, pool or bulk milk), individual or pooled swabs (vaginal, cervical, placental), feces, fetal tissue, and vaginal mucus from cattle, sheep, and goat. Up to 3 individual swab 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-C 071) for use in Germany for veterinary diagnostic procedures.

For veterinary use only.

Symbols

Symbol	Meaning
	Legal manufacturer
LOT	Lot number
	Use by date
	Temperature limitations for storage
	Handbook
REF	Catalog number
MAT	Material number
	Protect from light
	For samples from cattle, sheep, and goat

Quality control

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

Storage

The components of the bactotype C. burnetii 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 C. burnetii PCR Kit is a highly sensitive and specific solution for detection and quantification of DNA from *Coxiella burnetii* (*C. burnetii*) in samples from ruminant milk (quarter milk samples, pool or bulk milk), individual or pooled swabs (vaginal, cervical, placental), feces, fetal tissue and vaginal mucus from cattle, sheep and goat.

Q fever is a worldwide zoonotic disease caused by *C. burnetii*. This intracellular gram-negative bacterium can infect a large range of hosts (e.g., cattle, sheep, goat and humans).

Infections caused by *C. burnetii* are often subclinical infections in ruminants, but can also cause abortions and decreased reproductive efficiency.

In humans, *C. burnetii* may cause influenza-like illness, but also atypical pneumonia or hepatitis. An infection is thought to occur predominantly via aerosols generated by infected animals or animal products.

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 identified 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 detection of the accumulating product without the need to re-open the reaction tubes afterward.

The bactotype C. burnetii PCR Kit contains all of the necessary reagents for the detection and quantification of DNA from *Coxiella*

burnetii, including a positive and negative control, as well as an internal control and a DNA standard.

The internal control reduces the risk of false-negative results due to PCR inhibition or insufficient extraction of the sample.

The kit uses two specific primer/probe combinations:

- FAM™ fluorescence for DNA from *Coxiella burnetii*
- HEX™ fluorescence for the internal control (β-actin DNA, present within the sample)

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

DNA extraction

The bactotype C. burnetii PCR Kit can be used for detection and quantification of *Coxiella burnetii* DNA from cattle, sheep and goats in:

- milk (quarter milk samples, pool or bulk milk)
- individual or pooled swabs (vaginal, cervical, placental)
- feces
- fetal tissue
- vaginal mucus

Up to 3 individual swab samples can be tested in a pool.

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

Note: Specific pretreatments of the samples may be necessary.

Extraction based on magnetic beads:

- **IndiMag® Mastitis Kit** (for the extraction from milk samples; SP947757; formerly MagAttract® Mastitis Kit)
- **IndiMag Pathogen Kit** (SP947457; formerly MagAttract 96 cador® Pathogen Kit)
- **IndiMag Pathogen Kit w/o plastics** (SP947257; formerly MagAttract 96 cador Pathogen Kit w/o Plastics)

Extraction based on spin columns:

- **IndiSpin® Mastitis Kit** (for the extraction from milk samples; SP69805; formerly DNeasy® Mastitis Mini Kit)
- **IndiSpin Pathogen Kit** (SP54104, SP54106; formerly QIAamp® cador Pathogen Mini Kit)
- **IndiSpin QIAcube® HT Pathogen Kit** (SP54161; formerly cador Pathogen 96 QIAcube HT Kit)

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

For further information on automated and manual extraction of *Coxiella burnetii* 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
- Optional (for quantitative analysis): 0.1x TE buffer (pH 7-8) or DNase/RNase free water

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 5 µl of the Positive Control provided with

the bactotype C. burnetii 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 additional primer/probe set that detects a housekeeping gene (β-actin DNA) present within the sample.

Standard

The bactotype C. burnetii PCR Kit contains a DNA standard which allows quantitative analysis. A dilution series of the provided standard, followed by amplification in real-time PCR, enables quantification of an unknown sample.

Protocol: Real-time PCR for detection of DNA from *Coxiella burnetii*

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.

Preparation of the standard for quantitative analysis (optional)

Dilute the provide Cox Standard in a series prior to PCR setup. Please follow the scheme provided in Table 1, preferably using 0.1x TE buffer, pH7-8, or DNase/RNase-free water. Freeze the dilution series at -30°C to -15°C (in aliquots if used only intermittently). Avoid repeated thawing and freezing (>2x), as this may reduce reactivity.

Table 1. Dilution series of Cox Standard

	Dilution	**Copies/ ml**	**Preparation of dilution series**
1	undiluted	2 x 10^7 cp/ ml	
2	10^{-1}	2 x 10^6 cp/ ml	10 µl $\text{Standard}_{\text{undiluted}}$ + 90 µl 0.1x TE
3	10^{-2}	2 x 10^5 cp/ ml	10 µl Standard 10^{-1} + 90 µl 0.1x TE
4	10^{-3}	2 x 10^4 cp/ ml	10 µl Standard 10^{-2} + 90 µl 0.1x TE
5	10^{-4}	2 x 10^3 cp/ ml	10 µl Standard 10^{-3} + 90 µl 0.1x TE

* cp = copies

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 2).

   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.

   Optional (quantitative analysis): Use 5 µl of each dilution step of the prepared Cox Standard.

Table 2. 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 3.

Table 3. Filter settings for the reporter

Pathogen/ Internal Control	Reporter
Coxiella burnetii	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 instruments (Applied Biosystems®)

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

Note: This protocol can also be used if running bactotype Mastitis assays simultaneously in the same PCR thermal cycler (i.e., bactotype Mastitis HP3, bactotype Mastitis HP2+, bactotype Mastitis Screening, bactotype Mastitis Env).

Table 4. Real-time PCR protocol

Step	Temperature	Time	Number of cycles
Initial Activation	95°C	5 min	1
2-step cycling			
Denaturation	95°C	10 s	40
Annealing/Extension*	57°C	30 s	

* Fluorescence data collection.
Approximate run time 58 min (AriaMx Real-Time PCR System, Agilent)

Data analysis and interpretation

Interpretation of results (qualitative analysis)

For the assay to be valid the FAM and HEX fluorescence of the Positive Control must give a signal with a C_T[1] < 35. The Negative Control must give no fluorescence signal.

The following results are possible if working with unknown samples. The possible sample results are also summarized in Table 5 on page 16.

The sample is positive for *Coxiella burnetii*, 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 both the FAM and the HEX channel.
- The Negative Control does not yield a signal in the FAM and HEX channel.

Note that very high concentrations of *Coxiella burnetii* 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 *Coxiella burnetii*, and the assay is valid, if the following criteria are met:

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

[1] C_T, 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 both the FAM and the HEX channel.

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

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

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

If no signal is detected in both the FAM (pathogen) and the HEX (Internal Control) channel, the result is inconclusive. The absence of a signal for the housekeeping gene 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 5. Results interpretation table[1]

FAM	HEX	Sample result
X	X	positive for ***Coxiella burnetii***
X		strong positive for ***Coxiella burnetii***
	X	negative
		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 the FAM and the HEX channel. The Negative Control must yield no signal in the FAM and the HEX channel. For a complete explanation of possible sample results please refer to “Data analysis and interpretation” on page 15.

Interpretation of results (quantitative analysis)

Assign quantification values (copies/ ml) to each dilution point of the Cox Standard in the software of the thermal cycler. For the quantitative test to be valid, the criteria for the qualitative analysis as well as the following, additional criteria for quantitative analysis must be met:

- at least 4 out of 5 dilution steps of the Cox Standard should give a signal in the FAM channel,
- PCR correlation coefficient: $R^2 > 0.96$, and
- PCR efficiency: 85% - 115% (see thermal cycler software analysis).

Calculation of the concentration in the sample

Use the real-time thermal cycler software to calculate the amount of *C. burnetii* target quantity for each sample using the quantification values for each dilution point of the Cox Standard (Table 6).

Table 6: Cox Standard quantification values

No	copies/ ml	GE*/ ml
1	2×10^7 cp/ ml	10^6 GE/ ml
2	2×10^6 cp/ ml	10^5 GE/ ml
3	2×10^5 cp/ ml	10^4 GE/ ml
4	2×10^4 cp/ ml	10^3 GE/ ml
5	2×10^3 cp/ ml	10^2 GE/ ml

* GE = genome equivalent (20 copies = 1 GE); cp = copies

Equation for conversion target copy number into GE units

GE/ ml = target copies per ml / 20

Example for calculation of the concentration in the original sample (liquid sample)

The calculation is based on the quantification of the *C. burnetii* DNA sample (copies/ ml), the volume of the sample for extraction (SV) expressed in µl and the elution volume of the extracted method (EV) expressed in µl.

Copies/ ml = PCR quantification [copies/ ml] x (EV [µl] / SV [µl])

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.

Limited License Agreement for bactotype C. burnetii 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. INDICAL 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.indical.com. Some of these additional protocols have been provided by INDICAL users for INDICAL users. These protocols have not been thoroughly tested or optimized by INDICAL. INDICAL neither guarantees them nor warrants that they do not infringe the rights of third-parties.

2. Other than expressly stated licenses, INDICAL makes no warranty that this kit and/or its use(s) do not infringe the rights of third-parties.

3. This kit and its components are licensed for one-time use and may not be reused, refurbished, or resold.

4. INDICAL specifically disclaims any other licenses, expressed or implied other than those expressly stated.

5. The purchaser and user of the kit agree not to take or permit anyone else to take any steps that could lead to or facilitate any acts prohibited above. INDICAL may enforce the prohibitions of this Limited License Agreement in any Court, and shall recover all its investigative and Court costs, including attorney fees, in any action to enforce this Limited License Agreement or any of its intellectual property rights relating to the kit and/or its components.

For updated license terms, see www.indical.com.

Trademarks: bactotype®, cador®, cattletype®, flocktype®, IndiMag®, IndiSpin®, pigtype®, virotype® (INDICAL BIOSCIENCE GmbH); MagAttract®, QIAamp®, QIAcube® (QIAGEN GmbH); Applied Biosystems® (Applied Biosystems); FAM™, HEX™, JOE™, ROX™ (Life Technologies Corporation); Eppendorf® (Eppendorf-Netheler-Hinz GmbH). Licensed probes manufactured by Integrated DNA Technologies, Inc. Registered names, trademarks, etc. used in this document, even when not specifically marked as such, are not to be considered unprotected by law.

HB-2510-EN-001 © 2019 INDICAL BIOSCIENCE GmbH, all rights reserved.

Change index

Handbook	Version	Change
HB-2510-EN-001	May 2019	Product launch

Ordering: **www.indical.com/contact**
Technical Support: **support@indical.com**
Website: **www.indical.com**