

***cador*[®] T. equigenitalis PCR Reagent Handbook**



96 (catalog no. 285015)

For identification of DNA from *Taylorella equigenitalis*

REF

285015

For research use only.

USA/Canada: not for use in diagnosis of disease in animals.



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Reagent Contents

<i>cador</i> <i>T. equigenitalis</i> PCR Reagent	(96)
Catalog no.	285015
Number of reactions	96
T. equi Master Mix (blue cap)	8 x 12 reactions
T. equi Positive Control (red cap)	2 x 200 µl
T. equi Internal Control (green cap) IC	2 x 1000 µl
T. equi Mg-Sol (yellow cap) Mg-Sol	1 x 1000 µl

Intended Use

The *cador* *T. equigenitalis* PCR Reagent is intended for identification of *Taylorella equigenitalis* DNA using polymerase chain reaction (PCR).

For research use only. Not for use in veterinary diagnostic procedures. No claim or representation is intended to provide information for the diagnosis, prevention, or treatment of a veterinary disease.

All due care and attention should be exercised in the handling of the products. We recommend all users of QIAGEN products to adhere to the NIH guidelines that have been developed for recombinant DNA experiments, or to other applicable guidelines.

Symbols



<N>

Contains reagents for <N> tests



Legal manufacturer



Lot number



Use by date



Temperature limitations for storage



Catalog number



Material number



Protect from light

IC

Internal Control

Mg-Sol

Magnesium solution

Storage

The components of the *cador* T. equigenitalis PCR Reagent should be stored at -15 to -30°C and are stable until the expiration date stated on the label. Repeated thawing and freezing ($>2\times$) should be avoided, as this may reduce assay sensitivity. If the reagents are to be used only intermittently, they should be frozen in aliquots. Storage at 2 – 8°C should not exceed a period of 5 hours.

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 *cador* T. equigenitalis PCR Reagent is tested against predetermined specifications to ensure consistent product quality.

Introduction

The *cador* *T. equigenitalis* PCR Reagent is a ready-to-use system for the identification of *T. equigenitalis* DNA using real-time PCR. The *T. equi* Master Mix contains reagents and enzymes for the specific amplification of a highly conserved region of the *T. equigenitalis* genome. The amplicons are identified by measuring the FAM™ fluorescence. In addition, the *cador* *T. equigenitalis* PCR Reagent contains a heterologous amplification system to identify possible PCR inhibition. This is determined by measuring the fluorescent signal in the HEX™ or JOE™ channel due to amplification of an internal control (*T. equi* Internal Control). An external positive control (*T. equi* Positive Control) is supplied.

Principle

DNA identification by the 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 via 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 identification of the accumulating product without having to re-open the reaction tubes after the PCR run.

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.

- Nucleic acids isolation kit (see “Bacterial DNA isolation”, page 9)
- Phosphate buffered saline (PBS, 0.1 M)
- Pipets (adjustable)
- Nuclease-free, aerosol-resistant pipet tips with filters
- Vortexer
- Desktop centrifuge with rotor for 2 ml reaction tubes
- Rotor-Gene® Q or 96-well plate real-time cyclers 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
- Ice or cooling block

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.

Bacterial DNA isolation

We recommend using the QIAamp® DNA Mini Kit (QIAGEN, cat. no. 51304 or 51306) for manual bacterial DNA purification. Purification of bacterial DNA using the QIAamp DNA Mini Kit is fully automatable on the QIAcube®. The innovative QIAcube uses advanced technology to process QIAGEN spin columns, enabling seamless integration of automated, low-throughput sample prep into your laboratory workflow. Sample preparation using the QIAcube follows the same steps as the manual procedure (i.e., lyse, bind, wash, and elute). For more information about the automated procedure, see the relevant protocol sheet available at www.qiagen.com/MyQIAcube. The *C. trachomatis* T. equigenitalis PCR Reagent is not compatible with phenol-based bacterial DNA isolation methods.

Internal control

An internal control (T. equi Internal Control) is supplied. This allows the user to control the DNA isolation procedure and to check for possible PCR inhibition. For this application, add the internal control to the sample lysate (or lysis buffer) at a ratio of 0.1 µl per 1 µl elution volume. For example, using the QIAamp DNA Mini Kit (QIAGEN), the DNA is eluted in 150 µl Buffer AE. Therefore 15 µl of the internal control should be added to the sample lysate. The quantity of internal control used depends only on the elution volume. The internal control should be added only to the mixture of lysis buffer and sample material or directly to the lysis buffer. The internal control must not be added to the sample material directly. If added to the lysis buffer, please note that the mixture of internal control and lysis buffer has to be freshly prepared and used immediately (storage of the mixture at room temperature or at 2–8°C for only a few hours may lead to internal control failure and a reduced extraction efficiency). Do not add the internal control directly to the sample material. The internal control can optionally be used just to check for possible PCR inhibition. For this application, add 1 µl of the internal control and 3.25 µl T. equi Mg-Sol, per reaction, directly to 16.75 µl T. equi Master Mix, as described in the protocol.

For each PCR assay, use 20 µl of the master mix produced as described above* and add 5 µl of the purified sample. If you are preparing a PCR run for several samples, increase the volume of T. equi Master Mix, T. equi Mg-Sol, and the internal control (T. equi Internal Control) according to the number of samples (see "Protocol: PCR", page 11).

*The increase in volume caused by adding the internal control is not taken into account when preparing the PCR assay. The sensitivity of the detection system is not impaired.

Protocol: PCR

Important points before starting

- Before beginning the procedure, read "Important Notes", page 9.
- Before beginning the procedure, read through the protocol and ensure that you are familiar with the operation of the chosen real-time PCR cycler.
- Make sure that at least one positive control (T. equi Positive Control), as well as one no template control (PCR grade water) is included per PCR run.

Things to do before starting

- Before each use, all reagents need to be thawed completely at room temperature (15–25°C), mixed (by pipetting repeatedly up and down or by pulse vortexing), and centrifuged briefly. Then place all reagents in a cooling block at 2–8°C or on ice.

Procedure

1. If you want to use the internal control to monitor the DNA isolation procedure and to check for possible PCR inhibition, follow step 1a. If you want to use the internal control exclusively to check for PCR inhibition, follow step 1b.
- 1a. The internal control has already been added in the isolation procedure (see "Internal control", page 10). In this case, prepare a master mix, in a cooling block at 2–

8°C or on ice, according to Table 1. Proceed with step 2.

The master mix typically contains all of the components needed for PCR except the sample. Prepare a volume of master mix at least 10% greater than that required for the total number of PCR assays to be performed.

Table 1. Preparation of master mix (internal control already added in the isolation procedure)

Number of samples	1	24	96
T. equi Master Mix	16.75 µl	402 µl	1608 µl
T. equi Mg-Sol	3.25 µl	78 µl	312 µl
T. equi Internal Control	0 µl	0 µl	0 µl
Total volume	20 µl	480 µl	1920 µl

1b. The internal control must be added directly to the T. equi Master Mix. In this case, prepare a master mix, in a cooling block at 2–8°C or on ice, according to Table 2. Proceed with step 2.

The master mix typically contains all of the components needed for PCR except the sample. Prepare a volume of master mix at least 10% greater than that required for the total number of PCR assays to be performed.

Table 2. Preparation of master mix (internal control not added in the isolation procedure)

Number of samples	1	24	96
T. equi Master Mix	16.75 μ l	402 μ l	1608 μ l
T. equi Mg-Sol	3.25 μ l	78 μ l	312 μ l
T. equi Internal Control	1 μ l	24 μ l	96 μ l
Total volume	21 μl*	504 μl*	2016 μl*

* The increase in volume caused by adding the internal control is not taken into account when preparing the PCR assay. The sensitivity of the detection system is not impaired.

- 2. Pipet 20 μ l of the master mix into each reaction tube. Then add 5 μ l of the eluate from the DNA isolation (see Table 3). Correspondingly, 5 μ l of the positive control (T. equi Positive Control) must be used as a positive control and 5 μ l of water (Water, PCR grade) as a no template control. Mix the solutions thoroughly by pipetting repeatedly up and down.**

The external positive control (T. equi Positive Control) is treated as a previously purified sample and the same volume is used (5 μ l). This positive control provides a positive reference signal.

Table 3. Preparation of reaction mix

Component	Volume
Master mix	20 μ l
Sample	5 μ l
Total volume	25 μ l

3. Close the reaction tubes with the corresponding caps and centrifuge for 30 s at 1780 x g (4000 rpm) to collect the prepared reaction volume in the bottom of the tube.
4. Set the filters for the reporter and quencher dyes in the software of your thermal cycler according to Table 4. Select the green and yellow channels on the Rotor-Gene Q.

Table 4. Filter settings for reporter and quencher

Pathogen/Internal Control	Reporter	Quencher
<i>T. equigenitalis</i>	FAM	TAMRA™
Internal control	HEX/JOE*	TAMRA
Passive reference†	ROX™	

* Use the option appropriate for your thermal cycler.

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

5. Run the real-time PCR protocol according to Table 5.

Table 5. Real-time PCR protocol

Temperature	Time	Number of cycles
95°C	5 min	1
95°C	15 s	45
60°C*	45 s	

* Fluorescence data collection.

Data Analysis and Interpretation

Interpretation of results

The following results (1a, 1b, or 1c) are possible.

- 1a. A fluorescent signal is identified in the FAM (green channel in the Rotor-Gene Q) channel.**
The result of the analysis is positive: The sample contains *T. equigenitalis* DNA.

In this case, the identification of a fluorescent signal in the HEX (yellow channel in the Rotor-Gene Q; internal control) channel is not necessary since high initial concentrations of *T. equigenitalis* DNA (positive FAM/green fluorescence signal) can lead to a reduced or missing fluorescence signal of the internal control due to competition.

- 1b. No fluorescent signal is identified in the FAM (green channel in the Rotor-Gene Q) channel. At the same time, a fluorescent signal from the internal control appears in the HEX channel (yellow channel in the Rotor-Gene Q). In the sample no *T. equigenitalis* DNA is identifiable. It can be considered negative.**

In the case of a negative *T. equigenitalis* PCR result, the identified signal of the internal control rules out the possibility of PCR inhibition.

- 1c. No fluorescent signal is identified in the FAM (green channel in the Rotor-Gene Q) channel or in the HEX**

channel (yellow channel in the Rotor-Gene Q).

No result can be concluded.

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

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).

Comments and suggestions

No FAM fluorescence signal with positive control (*T. equi* Positive Control)

- | | | |
|----|---|--|
| a) | The selected identification dye for PCR data analysis does not comply with the protocol | For data analysis, select the identification dye FAM for the analytical <i>T. equigenitalis</i> PCR and the identification dye HEX/JOE for the internal control PCR. |
| b) | Incorrect programming of the temperature profile | Compare the temperature profile with the protocol (see "Protocol: PCR", page 11). |
| c) | Incorrect storage conditions for one or more reagent components or reagents expired | Check the storage conditions and the expiration date (see the product label) of the reagents and use new reagents, if necessary. Make sure that reagent components are stored as described in "Storage", page 5. |

Weak or no signal of the *T. equi*. Internal Control (HEX/JOE fluorescence signal) and simultaneous absence of a FAM fluorescence signal for the specific *T. equigenitalis* PCR

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|----|--|--|
| a) | PCR conditions do not comply with the protocol | Check the PCR conditions (see "Protocol: PCR", page 11). |
|----|--|--|

Comments and suggestions

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|--|---|
| b) PCR inhibited | <p>Make sure that you use a recommended isolation method (see "Bacterial DNA isolation", page 9) and follow the manufacturer's instructions closely.</p> <p>Make sure that during the DNA isolation the recommended additional centrifugation step has been carried out before the elution in order to remove any residual ethanol (see "Bacterial DNA isolation", page 9).</p> |
| c) DNA lost during extraction | <p>If the internal control has been added to the extraction, the lack of a signal for the internal control can indicate loss of DNA during the extraction. Make sure that you use a recommended isolation method (see "Bacterial DNA isolation", page 9) and follow the manufacturer's instructions closely.</p> |
| d) Incorrect storage conditions for one or more reagent components or reagents expired | <p>Check the storage conditions and the expiration date (see the product label) of the reagents and use new reagents, if necessary. Make sure that reagent components are stored as described in "Storage", page 5.</p> |

Comments and suggestions

A FAM fluorescence signal of the analytical PCR with the no template control

- | | |
|--|---|
| a) Contamination during preparation of the PCR | <p>Repeat the PCR with new reagents in replicates.</p> <p>If possible, close the PCR tubes directly after addition of the sample to be tested.</p> <p>Make sure to pipet the positive control last.</p> <p>Make sure that work space and instruments are decontaminated at regular intervals.</p> |
| b) Contamination during extraction | <p>Repeat the extraction and PCR of the sample to be tested using new reagents.</p> <p>Make sure that work space and instruments are decontaminated at regular intervals.</p> |

Ordering Information

Product	Contents	Cat. no.
<i>cad</i> or T. equigenitalis PCR Reagent (96)	For 96 reactions: T. equi Master Mix, T. equi Positive Control, T. equi Internal Control, T. equi Mg-Sol	285015
Related products		
QIAamp DNA Mini Kit — For isolation of genomic, bacterial, parasite, or viral DNA		
QIAamp DNA Mini Kit (50)*	For 50 DNA preps: 50 QIAamp Mini Spin Columns, QIAGEN Proteinase K, Reagents, Buffers, Collection Tubes (2 ml)	51304
QIAamp DNA Mini Kit (250)*	For 250 DNA preps: 250 QIAamp Mini Spin Columns, QIAGEN Proteinase K, Reagents, Buffers, Collection Tubes (2 ml)	51306
QIAcube — for fully automated sample preparation using QIAGEN spin-column kits		
QIAcube (110 V) [†]	Robotic workstation for automated purification of nucleic acids or proteins using QIAGEN spin-column kits, 1-year warranty on parts and labor [§]	9001292 [†]
QIAcube (230 V) [‡]		9001293 [‡]

* Fully automatable on the QIAcube. See www.qiagen.com/MyQIAcube for protocols.

[†] US, Canada, and Japan.

[‡] Rest of world.

[§] Agreements for comprehensive service coverage are available; please inquire.

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Trademarks: QIAGEN®, QIAamp®, QIAcube®, *cador*®, Rotor-Gene® Q (QIAGEN Group); ABI PRISM®, FAM™, JOE™, HEX™, ROX™ (Applied Biosystems or its subsidiaries);

The purchase of this product allows the purchaser to use it for amplification and identification of nucleic acid sequences for laboratory use only. No general patent or other license of any kind other than this specific right of use from purchase is granted hereby.

Limited License Agreement for *cador* T. equigenitalis PCR Reagent

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