Kit contents

cador T. equigenitalis PCR Kit	**(24)**
Cat. no.	**CD285023**
Number of reactions	**24**
cador T. equi Master Mix (tube with blue cap), includes primers, probes and enzymes	2 x 12 reactions
cador T. equi Positive Control (tube with red cap)	1 x 200 µl
cador T. equi Internal Control (tube with green cap)	1 x 1000 µl
cador T. equi Mg-Sol (tube with yellow cap)	1 x 1000 µl
H_2O (PCR Grade Water, tube with white cap)	1 x 1000 µl
Handbook	1

Intended use

The cador T. equigenitalis PCR Kit is intended for the detection of DNA from *Taylorella equigenitalis* in samples from horses (genital swab, culture medium).

The kit is approved by the Friedrich-Loeffler-Institut and licensed in accordance with § 11 (2) of the German Animal Health Act (FLI-B 470) 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 equine samples

Quality control

In accordance with INDICAL's ISO-certified Quality Management System, each lot of cador T. equigenitalis PCR Kit is tested against predetermined specifications to ensure consistent product quality.

Storage

The components of the cador T. equigenitalis 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 cador T. equigenitalis PCR Kit is a ready-to-use system for the detection of DNA from *Taylorella equigenitalis* (*T. equigenitalis*).

Taylorella equigenitalis is the bacterium responsible for Contagious Equine Metritis (CEM). CEM is a highly contagious venereal disease of horses, with symptoms in mares ranging from temporary infertility to severe and purulent inflammation of the uterine lining. Infected stallions do not show any clinical symptoms.

The bacterium is usually spread through direct transmission during mating. Testing for *T. equigenitalis* is important to identify infected animals before breeding. The infected animals can then be treated with antibiotics and topical disinfection to eliminate the bacteria prior to breeding.

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 cador T. equigenitalis PCR Kit contains all of the necessary reagents for the detection of DNA from *Taylorella equigenitalis*, including a positive and negative control.

The T. equi Master Mix contains reagents and enzymes for the specific amplification of a highly conserved region of the *T. equigenitalis*

genome. In addition, the PCR Kit contains a heterologous amplification system to identify possible PCR inhibition. The detection limit of the analytical *T. equigenitalis* PCR is not reduced.

The kit uses two specific primer/probe combinations:

- FAM™ fluorescence for DNA from *Taylorella equigenitalis*
- HEX™/ JOE™ fluorescence for the internal control (T. equi Internal Control)

DNA extraction

The cador T. equigenitalis PCR Kit is intended for the detection of DNA from *Taylorella equigenitalis* in samples from horses (genital swab, culture medium).

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: The cador T. equigenitalis PCR Kit is not compatible with phenol-based bacterial DNA isolation methods.

Extraction based on magnetic beads:

- **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 Pathogen Kit** (SP54104, SP54106; formerly QIAamp® cador Pathogen Mini Kit)
- **IndiSpin QIAcube® HT Pathogen Kit** (SP54161; formerly cador Pathogen 96 QIAcube HT Kit)

Furthermore, the QIAamp DNA Mini Kit can be purchased directly from QIAGEN (cat. no. 51304 or 51306).

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 *T. equigenitalis* 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.

- PBS (Phosphate buffered saline; 0.1M)
- Pipets
- Nuclease-free, aerosol-resistant pipet tips with filters
- 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
- Sterile 1.5 ml Eppendorf® tubes
- Vortex mixer
- Benchtop centrifuge with rotor for 1.5 ml tubes
- Cooling device or ice
- 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 (H_2O, PCR grade) 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 cador T. equigenitalis PCR Kit to test for successful amplification of the target.

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 (e.g., 10 µl T. equi Internal Control when eluting in 100µl volume).

Note: The internal control should be added only to the mixture of lysis buffer and sample material or directly to the lysis buffer, but not directly to the sample. 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.

Optionally, the internal control can 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.

Protocol: Real-time PCR for detection of DNA from *Taylorella equigenitalis*

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 (H_2O, PCR grade) 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

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 11). 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**
cador T. equi Master Mix	16.75 µl	402 µl
cador T. equi Mg-Sol	3.25 µl	78 µl
cador T. equi Internal Control	0 µl	0 µl
Total volume	**20 µl**	**480 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
cador T. equi Master Mix	16.75 µl	402 µl
cador T. equi Mg-Sol	3.25 µl	78 µl
cador T. equi Internal Control	1 µl	24 µl
Total volume	**21 µl***	**504 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 µl of the Master Mix into each reaction tube. Then add 5 µl of the eluate from the DNA isolation (Table 3).**

   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 provided H_2O (PCR grade) instead of sample DNA.

Table 3. Preparation of reaction mix

Component	Volume
Master Mix	20 µl
Sample	5 µl
Total volume	**25 µl**

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 dyes in the software of your thermal cycler according to Table 4.**

Table 4. Filter settings for the reporter

Pathogen/ Internal Control	Reporter
Taylorella equigenitalis	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®)

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

Table 5. Real-time PCR protocol

Step	Temperature	Time	Number of cycles
Initial Activation	95°C	5 min	1
2-step cycling			
Denaturation	95°C	15 s	40
Annealing/Extension*	60°C	45 s	

* Fluorescence data collection.

Data analysis and interpretation

Interpretation of results

The following results 1a – 1c are possible if working with unknown samples. The possible sample results are also summarized in Table 6 on page 17.

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 DNA from *T. equigenitalis*.
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 this sample no DNA from *T. equigenitalis* 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.

Table 6. Results interpretation table

FAM	HEX	Sample result
X	X	positive for ***Taylorella equigenitalis***
X		strong positive for ***Taylorella equigenitalis***
	X	negative
		inconclusive

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 cador T. equigenitalis 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.

Change index

Handbook	Version	Change
HB-1591-EN-002	October 2019	INDICAL design