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virotype® PEDV/TGEV RT-PCR Kit Handbook

For simultaneous detection of RNA from PEDV and TGEV

Licensed in accordance with § 11 (2) of the German Animal Health Act
MA No.: FLI-C 001

REF 96 reactions (cat. no. VT283605)

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INDICAL BIOSCIENCE

Contents

Kit contents

virotype PEDV/TGEV RT-PCR Kit **Cat. no.** **Number of reactions**	**(96)** **VT283605** **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
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Intended use

The virotype PEDV/TGEV RT-PCR Kit is a real-time multiplex RT-PCR test kit intended for the simultaneous detection of RNA from *Porcine Epidemic Diarrhea Virus* (PEDV) and *Transmissible Gastroenteritis Virus* (TGEV) in feces, tissue, oral fluid and swab samples (individual and pooled) from swine.

The kit is approved by the Friedrich-Loeffler-Institut and licensed in accordance with § 11 (2) of the German Animal Health Act (FLI-C 001) 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 samples from swine

Quality control

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

Storage

The components of the virotype PEDV/TGEV RT-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 virotype PEDV/TGEV RT-PCR Kit is a highly sensitive and specific solution for simultaneous detection of RNA from *Porcine Epidemic Diarrhea Virus* (PEDV) and *Transmissible Gastroenteritis Virus* (TGEV) in feces, tissue, oral fluid and swab samples from swine.

PEDV is an enveloped RNA virus belonging to the *Alphacoronavirus* genus of the *Coronaviridae* family. The virus causes acute diarrhea and dehydration of swine resulting in significant mortality in neonatal piglets (up to 100%) and therefore substantial economic loss. Porcine Epidemic Diarrhea (PED) is a contagious disease transmitted mainly by the fecal-oral mode of transmission.

Due to the similarity of clinical signs of PED and Transmissible Gastroenteritis (TGE), both viruses can only be differentiated by laboratory tests. TGEV also belongs to the family of *Coronaviridae*.

The high sensitivity of the virotype PEDV/TGEV RT-PCR Kit allows reliable detection of the pathogen in individual and pooled swine samples.

Principle

Polymerase chain reaction (PCR) is based on the amplification of specific regions of the pathogen genome. In real-time RT-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 virotype PEDV/TGEV RT-PCR Kit contains all of the necessary reagents for the detection of PEDV RNA and TGEV RNA, including a Positive and Negative Control. With this kit, both reverse transcription and PCR are performed in one reaction tube, reducing the risk of contamination.

The kit uses three specific primer/probe combinations: one for the PEDV RNA yielding FAM™ fluorescence, one for TGEV RNA yielding Quasar® 670/Cy®5 fluorescence and one for a heterologous RNA control yielding HEX™ fluorescence. This internal control excludes the possibility of false-negative results.

A Positive Control serves to verify the functionality of the reaction mix for the amplification of the PEDV/TGEV RNA targets.

RNA extraction

The virotype PEDV/TGEV RT-PCR Kit can be used for simultaneous detection of PEDV RNA and TGEV RNA in feces, tissue, oral fluid and swab samples from swine. Due to the high sensitivity of the test, pools of up to 5 individual samples can be used, provided that the sample quality is good.

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

- QIAamp® cador® Pathogen Mini Kit
- cador® Pathogen 96 QIAcube® HT Kit
- MagAttract® 96 cador® Pathogen Kit

Furthermore, the following extraction kits can be purchased from QIAGEN GmbH

- QIAamp® Viral RNA Mini Kit
- RNeasy® Fibrous Tissue Mini Kit
- RNeasy® Mini Kit

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

RNA 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
- 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 viral RNA. 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 virotype PEDV/TGEV RT-PCR Kit to test for successful amplification of the target.

Internal control

For convenience, the Internal Control RNA is already included in the reagents provided. This eliminates the need to add the Internal Control to each sample separately during reaction setup. The Internal Control allows the user to monitor PCR inhibition.

Protocol: Real-time RT-PCR for detection of PEDV/TGEV RNA

Important points before starting

- Please read „Important notes“ on page 9 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.
- RNA is unstable. 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 20 µl of the Master Mix into each reaction tube. Then add 5 µl of the sample RNA (Table 1).

   Include positive and negative control reactions.

   Positive Control: Use 5 µl of the positive control (Positive Control) instead of sample RNA.

   Negative Control: Use 5 µl of the negative control (Negative Control) instead of sample RNA.

Table 1. 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 2.

   Note: Set a fixed gain of +4 in the green and +1 in the yellow and +5 in the red channels to ensure optimal fluorescence gains for the pathogen and the Internal Control assays when using the Rotor-Gene® Q.

Table 2. Filter settings for the reporter

Pathogen/ internal control	Reporter	Rotor-Gene Q
PEDV	FAM	green
TGEV	Quasar 670[1]/ Cy5	red
Internal Control	HEX/ JOE[2]	yellow
Passive reference[3]	ROX	-

1 Quasar 670 as reporter dye has an excitation/emission maxima of 644/670 nm, allowing detection in the same channel as Cy5 and therefore can be used with most real-time cyclers.
2 Use the option appropriate for your thermal cycler.
3 Internal reference for use on Applied Biosystems® 7500

4. Run the real-time RT-PCR protocol according to Table 3 if running only the virotype PEDV/TGEV RT-PCR Kit.

Table 3. Real-time RT-PCR protocol for PEDV/TGEV

Step	Temperature	Time	Number of cycles
Reverse Transcription	50°C	10 min	1
Initial Activation	95°C	10 min	1
2-step cycling			
Denaturation	95°C	15 s	40
Annealing/Extension*	60°C	45 s	

* Fluorescence data collection. Approximate run time 1h 46min (Rotor-Gene Q)

5. Run the real-time RT-PCR protocol according to Table 4 if running other virotype assays simultaneously in the same thermal cycler (i.e., virotype PRRSV, virotype Influenza A and/or virotype CSFV).

Table 4. Real-time RT-PCR protocol for simultaneous assays

Step	Temperature	Time	Number of cycles
Reverse Transcription	50°C	20 min	1
Initial Activation	95°C	15 min	1
3-step cycling			
Denaturation	95°C	30 s	
Annealing*	57°C	45 s	40
Extension	68°C	45 s	

* Fluorescence data collection. Approximate run time 2h 38min (Rotor-Gene Q)

Data analysis and interpretation

Interpretation of results

For the assay to be valid the FAM, Quasar 670/ Cy5 and HEX fluorescence of the Positive Control must give a signal with a C_T[1] < 35. The Negative Control must show a HEX fluorescence signal but no FAM or Quasar 670/ Cy5 fluorescence signal.

The following results are possible if working with unknown samples. The possible sample results are also summarized in Table 5 on page 17.

The sample is positive for PEDV, 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 sample yields no signal in the Quasar 670/ Cy5 channel.
- The Positive Control yields a signal in all channels.
- The Negative Control does not yield a signal in both the FAM and Quasar 670/ Cy5 channel.

Note that very high concentrations of PEDV RNA in the sample may lead to a reduced HEX signal or no HEX signal due to competition with the internal control.

[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 sample is positive for TGEV, and the assay is valid, if the following criteria are met:

- The sample yields a signal in both the Quasar 670/ Cy5 and HEX channel.
- The sample yields no signal in the FAM channel.
- The Positive Control yields a signal in all channels.
- The Negative Control does not yield a signal in both the FAM and Quasar 670/ Cy5 channel.

Note that very high concentrations of TGEV RNA 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 both PEDV and TGEV, 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 all channels.
- The Negative Control does not yield a signal in both the FAM and Quasar 670/ Cy5 channel.

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

- The sample yields no signal in the FAM, Quasar 670/ Cy5 and HEX channels.

If no signal is detected in the FAM, Quasar 670/ Cy5 and HEX channels, the result is inconclusive. The absence of a signal in the Internal Control indicates PCR inhibition and/or other malfunctions.

To check for inhibition, we recommend 1:5 dilution of the sample RNA in nuclease-free water.

Check that there is a fluorescence signal in the FAM and Quasar 670/ Cy5 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*

Sample result	FAM (PEDV)	Quasar 670/ Cy5 (TGEV)	HEX (IC)
PEDV positive	X		X
PEDV positive (strong positive)	X		
TGEV positive		X	X
TGEV positive (strong positive)		X	
PEDV/TGEV negative			X
inconclusive			

* 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, Quasar 670/ Cy5 and HEX channel. The negative control must yield no signal in the FAM and Quasar 670/ Cy5 channel. For a complete explanation of possible sample results please refer to “Data analysis and interpretation” on page 15.

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.

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Change index

Handbook	Version	Change
HB-1934-EN-003	Nov 2018	Amendment in „Interpretation of results“
HB-1934-002	May 2018	INDICAL design

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