November 2014

virotype® CSFV RT-PCR Kit Handbook

24 (catalog no. 281803)

96 (catalog no. 281805)

480 (catalog no. 281807)*

For detection of RNA from classical swine fever virus

Registered in accordance with § 17c of the German Law on Animal Diseases (FLI-B 517)

REF 281803, 281805, 281807*

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QIAGEN®

* Available only on request.

Sample & Assay Technologies

QIAGEN Sample and Assay Technologies

QIAGEN is the leading provider of innovative sample and assay technologies, enabling the isolation and detection of contents of any biological sample. Our advanced, high-quality products and services ensure success from sample to result.

QIAGEN sets standards in:

- Purification of DNA, RNA, and proteins
- Nucleic acid and protein assays
- microRNA research and RNAi
- Automation of sample and assay technologies

Our mission is to enable you to achieve outstanding success and breakthroughs. For more information, visit www.qiagen.com.

In addition, QIAGEN provides high-quality, easy-to-use, and sensitive molecular solutions to enable veterinary pathogen detection and animal pathogen research. The QIAGEN veterinary portfolio includes a broad range of pathogen-specific PCR-assays and an extensive and growing ELISA portfolio. For more information, visit www.qiagen.com/Animal-and-Veterinary-Testing.

Contents

Kit Contents

virotype CSFV RT-PCR Kit	(24)	(96)	(480)
Catalog no.	**281803**	**281805**	**281807***
Number of reactions	**24**	**96**	**480**
Master Mix (tube with orange cap) includes enzymes, primers, and probes	1 x 500 µl	2 x 980 µl	6 x 1625 µl
Positive Control (tube with red cap)	1 x 25 µl	1 x 70 µl	2 x 50 µl
Negative Control (tube with blue cap)	1 x 25 µl	1 x 70 µl	2 x 50 µl
Handbook	1	1	1

* Available only on request.

Intended Use

The *virotype* CSFV RT-PCR Kit is intended for the detection of RNA from classical swine fever virus (CSFV) in serum, plasma, EDTA - blood, and tissue samples from pigs and wild boar. The kit is approved by the Friedrich-Loeffler-Institut and registered in accordance with § 17c of the German Law on Animal Diseases (FLI-B 517) for use in Germany for veterinary diagnostic procedures. For veterinary use only.

Symbols

	Contains reagents for <N> tests
	Legal manufacturer
	Lot number
	Use by date
	Temperature limitations for storage
	Handbook
	Catalog number
	Material number
	Protect from light
	For pig and wild boar samples

Storage

The components of the *virotype* CSFV RT-PCR Kit should be stored at –15 to –30°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 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 *virotype* CSFV RT-PCR Kit is tested against predetermined specifications to ensure consistent product quality.

Introduction

The *virotype* CSFV RT-PCR Kit is a highly sensitive and specific solution for the detection of RNA from classical swine fever virus (CSFV) in samples from pigs and wild boar. Classical Swine Fever (CSF) is economically one of the most important viral infectious diseases of swine. CSF is widespread in domestic pig and wild boar populations. CSF is an internationally notifiable animal disease. The causative agent, classical swine fever virus (CSFV), is a single-stranded RNA virus and a member of the genus pestivirus which belongs to the *Flaviviridae* family like bovine viral diarrhea virus in cattle and border disease virus in sheep.

The high sensitivity of the *virotype* CSFV RT-PCR Kit allows the early detection of the pathogen in individual as well as in pooled blood samples and in individual and pooled tissue samples. The *virotype* CSFV RT-PCR Kit detects all known CSFV-strains. In rare cases the test may detect CSF vaccine virus. Positive results of animals from areas where CSFV vaccination is performed should therefore be verified.

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 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 afterward.

The *virotype* CSFV RT-PCR Kit contains all of the necessary reagents for the detection of CSFV 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.

An internal control excludes the possibility of false-negative results.

The kit uses two specific primer/probe combinations: one for CSFV RNA yielding FAM™ fluorescence and one for a housekeeping gene (β-actin mRNA) present within the sample yielding HEX™ fluorescence. A Positive Control serves to verify the functionality of the reaction mix for the amplification of the CSFV RNA target.

RNA extraction

The *virotype* CSFV RT-PCR Kit can be used for the detection of CSFV RNA from serum, plasma, EDTA - blood, and tissue samples from swine. Due to the high sensitivity of the test individual or pooled samples can be tested. For domestic pigs, up to 20 individual serum, plasma, or EDTA-blood samples or up to 10 tissue samples can be used. Furthermore, for wild boar, pools can consist of up to 10 serum, plasma, EDTA-blood, or tissue samples.

Prior to real-time RT-PCR, viral RNA must be extracted from the starting material. QIAGEN offers a range of products for RNA extraction from animal samples.

- QIAamp® *cador*® Pathogen Mini Kit

- QIAamp Viral RNA Mini Kit
- QIAamp MinElute® Virus Spin Kit
- RNeasy® Fibrous Tissue Mini Kit
- RNeasy Mini Kit
- RNeasy Lipid Tissue Mini Kit (for wild boar samples)

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
- Benchtop centrifuge with rotor for 1.5 ml tubes
- Cooling device or ice
- Rotor-Gene® Q or 96-well plate real-time cycler 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

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 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. 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, for example, the correct setup of the reaction mix. Use 5 µl of the Positive Control provided with the *virotype* CSFV RT-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 a second primer/probe set that detects a housekeeping gene present within the sample. This allows both extraction and amplification to be monitored.

Protocol: Real-time RT-PCR for identification of classical swine fever virus

Important points before starting

- Please read "Important Notes" on page 11 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 and quencher dyes in the software of your thermal cycler according to Table 2. Select the green and yellow channels on the Rotor-Gene Q.

Table 2. Filter settings for reporter and quencher

Pathogen/internal control	Reporter	Quencher
CSFV	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.

4. Run the real-time RT-PCR protocol according to Table 3 if running only the *virotype* CSFV RT-PCR Kit.

Table 3. Real-time RT-PCR protocol for CSFV

Temperature	Time	Number of cycles
45°C	10 min	1
95°C	10 min	1
95°C	15 s	
57°C*	30 s	40
72°C	35 s	

* Fluorescence data collection.

5. Run the real-time RT-PCR protocol according to Table 4 if running other *virotype* assays simultaneously (i.e., *virotype* BTVpan/8, *virotype* BVDV, *virotype* SBV, *virotype* PRRSV and/or *virotype* Influenza A).

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

Temperature	Time	Number of cycles
50°C	20 min	1
95°C	15 min	1
95°C	30 s	
57°C†	45 s	40
68°C	45 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 HEX channels with a C_T*<35. The Negative Control must give no signal.

The following results are possible if working with unknown samples. The possible sample results are also summarised in Table 5 on page 18.

The sample is positive for CSFV, and the assay is valid, if the following criteria are met:

- The sample yields a signal in both the FAM and HEX† channels
- The Positive Control yields a signal in both the FAM and HEX channels
- The Negative Control does not yield a signal in the FAM and HEX channels

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

* Threshold cycle (C_T) — cycle at which the amplification plot crosses the threshold, i.e., there is the first clearly detectable increase in fluorescence.

† Green and yellow on the Rotor-Gene Q.

The sample is negative for CSFV, and the assay is valid, if the following criteria are met:

- The sample yields a signal in the HEX channel but not in the FAM channel
- The Positive Control yields a signal in both the FAM and HEX channels
- The Negative Control does not yield a signal in the FAM and HEX channels

A positive HEX signal means that extraction and amplification were successful as the housekeeping gene within the sample is amplified. However, if the C_T value of the internal control is >35, pooled or individual samples could be partially inhibited. In such cases the respective individual samples should be diluted (e.g., diluted 1:5) in nuclease free water and retested.

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

- The sample yields no signal in the FAM and 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 RNA in nuclease free water, to repeat the RNA 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*

Sample result	Reporter	
	FAM (pathogen)	HEX (IC)
CSFV positive	X	X
CSFV positive (strong positive)	X	
CSFV negative		X
Inconclusive result		

* 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 HEX channels. The negative control must yield no signal in the FAM and HEX channels. For a complete explanation of possible sample results please refer to "Data Analysis and Interpretation" on page 16.

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).

Ordering Information

Product	Contents	Cat. no.
virotype CSFV RT-PCR Kit (24)	For 24 reactions: Master Mix, Positive Control, Negative Control	281803
virotype CSFV RT-PCR Kit (96)	For 96 reactions: Master Mix, Positive Control, Negative Control	281805
virotype CSFV RT-PCR Kit (480)*	For 480 reactions: Master Mix, Positive Control, Negative Control	281807
Related products		
bactotype MAP PCR Kit (24)†	For 24 reactions: Master Mix, Internal Control DNA, Positive Control, Negative Control	285903
bactotype Mycoplasma Mg/Ms PCR Kit (96)†	For 96 reactions: Master Mix, Positive Control, Negative Control	288105
virotype ASFV PCR Kit (96)	For 96 reactions: Master Mix, Positive Control, Negative Control	281905
virotype BTV RT-PCR Kit (96)†	For 96 reactions: PCR Mix, Enzyme Mix, Positive Control, Negative Control	280435

*Available only on request.
† Other kit sizes are available; see www.qiagen.com.

Product	Contents	Cat. no.
virotype BTV pan/8 RT-PCR Kit (96)*	For 96 reactions: Master Mix, Positive Control, Negative Control	280445
virotype BVDV RT-PCR Kit (96)*	For 96 reactions: PCR Mix, Enzyme Mix, Positive Control, Negative Control	280375
virotype SBV RT-PCR Kit (96)*	For 96 reactions: Master Mix, Positive Control, Negative Control	281605
virotype PRRSV RT-PCR Kit (96)*	For 96 reactions: Master Mix, Positive Control, Negative Control	282305
virotype Influenza A RT-PCR Kit (96)*	For 96 reactions: Master Mix, Positive Control, Negative Control	282605
QIAamp *cador* Pathogen Mini Kit (50)*	For 50 preps: 50 QIAamp Mini Spin Columns, Carrier RNA, Proteinase K, Collection Tubes (2 ml), RNase-free Buffers	54104

* Other kit sizes are available; see www.qiagen.com.

Product	Contents	Cat. no.
QIAamp Viral RNA Mini Kit (50)*	For 50 RNA preps: 50 QIAamp Mini Spin Columns, carrier RNA, Collection Tubes (2 ml), RNase-free buffers	52904
QIAamp Viral MinElute Virus Spin Kit (50)	For 50 preps: 50 QIAamp MinElute Columns, QIAGEN Protease, carrier RNA, buffers, Collection Tubes (2 ml)	57704
RNeasy Fibrous Tissue Mini Kit (50)	For 50 preps: 50 RNeasy Mini Spin Columns, Collection Tubes (1.5 ml and 2 ml), Proteinase K, RNase-free DNase I, RNase-free Reagents and Buffers	74704
RNeasy Mini Kit (50)*	For 50 preps: 50 RNeasy Mini Spin Columns, Collection Tubes (1.5 ml and 2 ml), RNase-free Reagents and Buffers	74104
RNeasy Lipid Tissue Mini Kit (50)	For 50 preps: 50 RNeasy Mini Spin Columns, Collection Tubes (1.5 ml and 2 ml), QIAzol Lysis Reagent, RNase-free Reagents and Buffers	74804

* Other kit sizes are available; see www.qiagen.com.

Product	Contents	Cat. no.
Rotor-Gene Q 5plex Platform	Real-time PCR cycler with 5 channels (green, yellow, orange, red, crimson), laptop computer, software, accessories, 1-year warranty on parts and labor	9001570

QIAGEN offer a range of ELISA kits and real-time PCR and real-time RT-PCR kits for the detection of animal pathogens. Visit www.qiagen.com/Animal-and-Veterinary-Testing for more information about the *bactotype*®, *cador* ®, *cattletype*®, *flocktype*®, *pigtype*®, and *virotype* products.

For up-to-date licensing information and product-specific disclaimers, see the respective QIAGEN kit handbook or user manual. QIAGEN kit handbooks and user manuals are available at www.qiagen.com or can be requested from QIAGEN Technical Services or your local distributor.

Notes

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