November 2014

virotype® PRRSV RT-PCR Kit Handbook

24 (catalog no. 282303)

96 (catalog no. 282305)

480 (catalog no. 282307)*

For detection of RNA from porcine reproductive and respiratory syndrome virus

REF 282303, 282305, 282307*

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* Available only on request.

Sample & Assay Technologies

QIAGEN Sample and Assay Technologies

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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

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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* PRRSV RT-PCR Kit**	**(24)**	**(96)**	**(480)**
Catalog no.	**282303**	**282305**	**282307***
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* PRRSV RT-PCR Kit is intended for the simultaneous detection of the North American (NA), European (EU) genotypes, and the highly pathogenic (HP) strain of the NA genotype, of porcine reproductive and respiratory syndrome virus (PRRSV) RNA. RNA can be detected in swine blood, serum, tissue, bronchial swabs, bronchial lavage, saliva, semen samples, and cell culture supernatant samples. 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 swine samples

Storage

The components of the *virotype* PRRSV 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* PRRSV RT-PCR Kit is tested against predetermined specifications to ensure consistent product quality.

Introduction

The *virotype* PRRSV RT-PCR Kit is a highly sensitive solution for the safe and simultaneous detection of the North American (NA) and European (EU) genotypes of porcine reproductive and respiratory syndrome virus (PRRSV) in samples from swine. It can also differentiate the Highly Pathogenic (HP) strain of the NA genotype.

Infections with PRRS virus in swine are very prevalent and economically very important for the swine industry. PRRSV is a RNA virus that belongs to the order Nidovirales, family Arteriviridae. PRRS viruses are classified into the European (EU/I) and the North American (NA/II) genotype. PRRS virus infection can cause respiratory disease in piglets and reproductive failure in pregnant sows. Since 2006 a highly pathogenic NA strain of PRRSV (HP PRRSV) emerged which is characterized by high fever and high mortality in swine of all ages.

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* PRRSV RT-PCR Kit contains all of the necessary reagents for the detection of PRRSV 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 *virotype* PRRSV RT-PCR Kit uses four specific primer/probe combinations: one for the RNA of the EU genotype yielding FAM™, one for RNA of the NA genotype yielding Texas Red, one for RNA of the HP strain yielding Cy5™, 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 PRRSV RNA targets.

RNA extraction

The *virotype* PRRSV RT-PCR Kit can be used for the detection of PRRSV RNA from swine blood, serum, tissue, bronchial swabs, bronchial lavage, saliva, semen samples, and cell culture supernatant. Due to the high sensitivity of the test, pools of up to 5 individual samples and collective samples of saliva may be analyzed.

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
- 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
- Benchtop centrifuge with rotor for 1.5 ml tubes
- Cooling device or ice
- 96-well plate real-time cycler with appropriate fluorescent channels
- Appropriate software for chosen 96-well plate cycler
- Strip Tubes and Caps, 0.1 ml, 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* PRRSV 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 another 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 porcine reproductive and respiratory syndrome 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 dyes in the software of your chosen thermal cycler according to Table 2.

Table 2. Filter settings for reporter

Pathogen/internal control	Reporter
EU genotype	FAM
NA genotype	Texas Red/ROX™*
HP strain	Cy5
Internal Control	HEX/JOE™*

* Use the option appropriate for your thermal cycler.

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

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

Temperature	Time	Number of cycles
45°C	10 min	1
95°C	10 min	1
95°C	15 s	
56°C*	30 s	40
72°C	30 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* CSFV, *virotype* SBV 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 FAM, Texas Red/ROX, Cy5, and HEX fluorescence of the Positive Control must give a signal with a C_T*<35. The Negative Control may show a HEX fluorescence signal but no FAM, Texas Red/ROX, and Cy5 fluorescence signal.

The following results are possible if working with unknown samples. The possible sample results are also summarised in Table 5 on page 19.

The sample is positive for PRRSV EU genotype, 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 all channels

Note that very high concentrations of PRRSV 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 positive for PRRSV NA genotype, and the assay is valid, if the following criteria are met:

- The sample yields a signal in both the Texas Red/ROX and HEX channels
- The Positive Control yields a signal in all channels

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

Note that very high concentrations of PRRSV 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 positive for both the PRRSV EU and NA genotypes, and the assay is valid, if the following criteria are met:

- The sample yields a signal in the FAM, Texas Red/ROX, and HEX channels
- The Positive Control yields a signal in all channels

Note that very high concentrations of PRRSV 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 positive for the HP strain, and the assay is valid, if the following criteria are met:

- The sample yields a signal in the Cy5, Texas Red/ROX, and HEX channels
- The Positive Control yields a signal in all channels

It is not possible to exclude the presence of the PRRSV NA genotype in addition to the PRRSV HP strain.

Note that very high concentrations of PRRSV 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 PRRSV, 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

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 any of the fluorescence channels

The PCR was inhibited or the sample extraction was incorrect. It is recommended to retest the respective individual samples in nuclease free water (e.g., diluted 1:5), 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 all channels for the positive control reaction (Positive Control). Absence of a signal for the Positive Control indicates an error, which could be due to RNA extraction failure, incorrect setup of the reaction mix, or incorrect cycling conditions.

Repeat RNA extraction or repeat the whole procedure starting with new sample material.

Table 5. Results interpretation table*

Fluorescence signal	Pathogen genotype/strain					
	EU	**NA**	**EU + NA**	**NA + HP**	**Negative**	**Invalid**
FAM	X		X			
Texas Red/ROX		X	X	X		
Cy5				X		
HEX	(X)	(X)	(X)	(X)	X	

* 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, HEX, Cy5, and Texas Red/ROX channels. The negative control must yield no signal in the FAM, Texas Red/ROX, and Cy5 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 PRRSV RT-PCR Kit (24)	For 24 reactions: Master Mix, Positive Control, Negative Control	282303
virotype PRRSV RT-PCR Kit (96)	For 96 reactions: Master Mix, Positive Control, Negative Control	282305
virotype PRRSV RT-PCR Kit (480)*	For 480 reactions: Master Mix, Positive Control, Negative Control	282307
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 CSFV RT-PCR Kit (96)*	For 96 reactions: Master Mix, Positive Control, Negative Control	281805
virotype SBV RT-PCR Kit (96)*	For 96 reactions: Master Mix, Positive Control, Negative Control	281605
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
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

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

Product	Contents	Cat. no.
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

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.

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

Notes

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Notes

The purchase of this product allows the purchaser to use it for amplification and detection of nucleic acid sequences for providing veterinary in vitro diagnostics. 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 *virotype* PRRSV RT-PCR Kit

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