virotype® PRRSV RT-PCR Kit Handbook

For detection of RNA from porcine reproductive and respiratory syndrome virus

REF 282303, 282305, 282307*

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* Available only on request.
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

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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.
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**Kit Contents**

<table>
<thead>
<tr>
<th>virotype PRRSV RT-PCR Kit</th>
<th>(24)</th>
<th>(96)</th>
<th>(480)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Catalog no.</td>
<td>282303</td>
<td>282305</td>
<td>282307*</td>
</tr>
<tr>
<td>Number of reactions</td>
<td>24</td>
<td>96</td>
<td>480</td>
</tr>
<tr>
<td>Master Mix (tube with orange cap)</td>
<td>1 x 500 μl</td>
<td>2 x 980 μl</td>
<td>6 x 1625 μl</td>
</tr>
<tr>
<td>includes enzymes, primers, and probes</td>
<td>1 x 25 μl</td>
<td>1 x 70 μl</td>
<td>2 x 50 μl</td>
</tr>
<tr>
<td>Positive Control (tube with red cap)</td>
<td>1 x 25 μl</td>
<td>1 x 70 μl</td>
<td>2 x 50 μl</td>
</tr>
<tr>
<td>Negative Control (tube with blue cap)</td>
<td>1 x 25 μl</td>
<td>1 x 70 μl</td>
<td>2 x 50 μl</td>
</tr>
<tr>
<td>Handbook</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
</tbody>
</table>

* 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

<table>
<thead>
<tr>
<th>Component</th>
<th>Volume</th>
</tr>
</thead>
<tbody>
<tr>
<td>Master Mix</td>
<td>20 μl</td>
</tr>
<tr>
<td>Sample</td>
<td>5 μl</td>
</tr>
<tr>
<td><strong>Total volume</strong></td>
<td><strong>25 μl</strong></td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Pathogen/internal control</th>
<th>Reporter</th>
</tr>
</thead>
<tbody>
<tr>
<td>EU genotype</td>
<td>FAM</td>
</tr>
<tr>
<td>NA genotype</td>
<td>Texas Red/ROX™*</td>
</tr>
<tr>
<td>HP strain</td>
<td>Cy5</td>
</tr>
<tr>
<td>Internal Control</td>
<td>HEX/JOE™*</td>
</tr>
</tbody>
</table>

* 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

<table>
<thead>
<tr>
<th>Temperature</th>
<th>Time</th>
<th>Number of cycles</th>
</tr>
</thead>
<tbody>
<tr>
<td>45°C</td>
<td>10 min</td>
<td>1</td>
</tr>
<tr>
<td>95°C</td>
<td>10 min</td>
<td>1</td>
</tr>
<tr>
<td>95°C</td>
<td>15 s</td>
<td></td>
</tr>
<tr>
<td>56°C*</td>
<td>30 s</td>
<td>40</td>
</tr>
<tr>
<td>72°C</td>
<td>30 s</td>
<td></td>
</tr>
</tbody>
</table>

* 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

<table>
<thead>
<tr>
<th>Temperature</th>
<th>Time</th>
<th>Number of cycles</th>
</tr>
</thead>
<tbody>
<tr>
<td>50°C</td>
<td>20 min</td>
<td>1</td>
</tr>
<tr>
<td>95°C</td>
<td>15 min</td>
<td>1</td>
</tr>
<tr>
<td>95°C</td>
<td>30 s</td>
<td></td>
</tr>
<tr>
<td>57°C†</td>
<td>45 s</td>
<td>40</td>
</tr>
<tr>
<td>68°C</td>
<td>45 s</td>
<td></td>
</tr>
</tbody>
</table>

† 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*

<table>
<thead>
<tr>
<th>Fluorescence signal</th>
<th>EU</th>
<th>NA</th>
<th>EU + NA</th>
<th>NA + HP</th>
<th>Negative</th>
<th>Invalid</th>
</tr>
</thead>
<tbody>
<tr>
<td>FAM</td>
<td>X</td>
<td></td>
<td>X</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Texas Red/ROX</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cy5</td>
<td></td>
<td></td>
<td></td>
<td>X</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HEX</td>
<td>(X)</td>
<td>(X)</td>
<td>(X)</td>
<td>(X)</td>
<td>X</td>
<td></td>
</tr>
</tbody>
</table>

* 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

<table>
<thead>
<tr>
<th>Product</th>
<th>Contents</th>
<th>Cat. no.</th>
</tr>
</thead>
<tbody>
<tr>
<td>virotypetype PRRSV RT-PCR Kit (24)</td>
<td>For 24 reactions: Master Mix, Positive Control, Negative Control</td>
<td>282303</td>
</tr>
<tr>
<td>virotypetype PRRSV RT-PCR Kit (96)</td>
<td>For 96 reactions: Master Mix, Positive Control, Negative Control</td>
<td>282305</td>
</tr>
<tr>
<td>virotypetype PRRSV RT-PCR Kit (480) *</td>
<td>For 480 reactions: Master Mix, Positive Control, Negative Control</td>
<td>282307</td>
</tr>
</tbody>
</table>

### Related products

| bactotypetype MAP PCR Kit (24) † | For 24 reactions: Master Mix, Internal Control DNA, Positive Control, Negative Control | 285903   |
| bactotypetype Mycoplasma Mg/Ms PCR Kit (96) † | For 96 reactions: Master Mix, Positive Control, Negative Control | 288105   |
| virotypetype ASFV PCR Kit (96) | For 96 reactions: Master Mix, Positive Control, Negative Control | 281905   |
| virotypetype 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.
<table>
<thead>
<tr>
<th>Product</th>
<th>Contents</th>
<th>Cat. no.</th>
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</thead>
<tbody>
<tr>
<td>viroteype BTV pan/8 RT-PCR Kit (96)*</td>
<td>For 96 reactions: Master Mix, Positive Control, Negative Control</td>
<td>280445</td>
</tr>
<tr>
<td>viroteype BVDV RT-PCR Kit (96)*</td>
<td>For 96 reactions: PCR Mix, Enzyme Mix, Positive Control, Negative Control</td>
<td>280375</td>
</tr>
<tr>
<td>viroteype CSFV RT-PCR Kit (96)*</td>
<td>For 96 reactions: Master Mix, Positive Control, Negative Control</td>
<td>281805</td>
</tr>
<tr>
<td>viroteype SBV RT-PCR Kit (96)*</td>
<td>For 96 reactions: Master Mix, Positive Control, Negative Control</td>
<td>281605</td>
</tr>
<tr>
<td>viroteype Influenza A RT-PCR Kit (96)*</td>
<td>For 96 reactions: Master Mix, Positive Control, Negative Control</td>
<td>282605</td>
</tr>
<tr>
<td>QIAamp cador Pathogen Mini Kit (50)*</td>
<td>For 50 preps: 50 QIAamp Mini Spin Columns, Carrier RNA, Proteinase K, Collection Tubes (2 ml), RNase-free Buffers</td>
<td>54104</td>
</tr>
<tr>
<td>QIAamp Viral RNA Mini Kit (50)*</td>
<td>For 50 RNA preps: 50 QIAamp Mini Spin Columns, carrier RNA, Collection Tubes (2 ml), RNase-free buffers</td>
<td>52904</td>
</tr>
</tbody>
</table>

* Other kit sizes are available; see [www.qiagen.com](http://www.qiagen.com).
<table>
<thead>
<tr>
<th>Product</th>
<th>Contents</th>
<th>Cat. no.</th>
</tr>
</thead>
<tbody>
<tr>
<td>RNeasy Fibrous Tissue</td>
<td>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</td>
<td>74704</td>
</tr>
<tr>
<td>Kit (50)</td>
<td>For 50 preps: 50 RNeasy Mini Spin Columns, Collection Tubes (1.5 ml and 2 ml), RNase-free Reagents and Buffers</td>
<td>74104</td>
</tr>
<tr>
<td>RNeasy Mini Kit (50)*</td>
<td>For 50 preps: 50 RNeasy Mini Spin Columns, Collection Tubes (1.5 ml and 2 ml), RNase-free Reagents and Buffers</td>
<td></td>
</tr>
</tbody>
</table>

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](http://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](http://www.qiagen.com) or can be requested from QIAGEN Technical Services or your local distributor.

* Other kit sizes are available; see [www.qiagen.com](http://www.qiagen.com).
Notes
Limited License Agreement for virotype PRRSV RT-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. QIAGEN 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.qiagen.com. Some of these additional protocols have been provided by QIAGEN users for QIAGEN users. These protocols have not been thoroughly tested or optimized by QIAGEN. QIAGEN neither guarantees them nor warrants that they do not infringe the rights of third-parties.

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