August 2015

# *virotype*® ASFV PCR Kit Handbook

24 (catalog no. 281903)

96 (catalog no. 281905)

## For detection of DNA from African swine fever virus (ASFV)

Licensed in accordance with § 11 (2) of the German Animal Health Act (FLI-B 670)

REF 281903, 281905

QIAGEN Leipzig GmbH, Deutscher Platz 5b,
04103 Leipzig, Germany

Sample to Insight

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# Contents

# Kit Contents

| ***virotype* ASFV PCR Kit** | **(24)** | **(96)** |
|---|---|---|
| **Catalog no.** | **281903** | **281905** |
| **Number of reactions** | **24** | **96** |
| Master Mix (tube with orange cap), includes enzymes, primers and probes | 1 x 500 µl | 2 x 980 µl |
| Positive Control (tube with red cap) | 1 x 25 µl | 1 x 70 µl |
| Negative Control (tube with blue cap) | 1 x 25 µl | 1 x 70 µl |
| Handbook | 1 | 1 |

# Intended Use

The *virotype* ASFV PCR Kit is intended for the detection of DNA from African swine fever virus (ASFV) in serum, plasma, EDTA-blood, tissue, and swab samples from pigs and wild boar.

The kit is approved by the Friedrich-Loeffler-Institut and licensed in accordance with § 11 (2) of the German Animal Health Act (FLI-B 670) for use in Germany for veterinary diagnostic procedures.

**For veterinary use only.**

# Symbols

| | |
|---|---|
|  <N> | 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* ASFV 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 online in convenient and compact PDF format at **www.qiagen.com/safety** where you can find, view, and print the MSDS 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* ASFV PCR Kit is tested against predetermined specifications to ensure consistent product quality.

# Introduction

The *virotype* ASFV PCR Kit is a highly sensitive and specific solution for the detection of DNA from African swine fever virus (ASFV) in samples from pigs and wild boar.

African swine fever (ASF) is one of the most important infectious viral diseases of swine of all ages and causes a wide range of clinical signs characterized by a high rate of morbidity and mortality. The disease is notifiable to the World Organization for Animal Health (OIE).

The causative agent is a double-stranded DNA virus belonging to the family *Asfarviridae*, genus *Asfivirus*. ASF virus can be transmitted by vectors (soft ticks of the genus *Ornithodoros*) therefore classified as *Arbovirus* (arthropod-borne virus).

The high sensitivity of the *virotype* ASFV PCR Kit allows early detection of the pathogen in individual as well as in pooled samples.

## 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 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 detection of the accumulating product without the need to re-open the reaction tubes afterward.

The *virotype* ASFV PCR Kit contains all of the necessary reagents for the detection of ASFV DNA, including a positive and negative control.

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

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

## DNA extraction

The *virotype* ASFV PCR Kit can be used for the detection of ASFV DNA from serum, plasma, EDTA-blood, tissue, and swab samples from swine. Due to the high sensitivity of the test individual or pooled samples can be tested. Pools of up to 20 individual serum,

plasma, EDTA-blood, or tissue samples can be used, provided that the sample quality is good. It is recommended to test dead wildlife samples on an individual basis.

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

- QIAamp® Viral RNA Mini Kit*
- QIAamp *cador*® Pathogen Mini Kit
- DNeasy® Blood & Tissue Kit
- QIAamp DNA Blood Mini Kit

If real-time PCR is not performed immediately after extraction, store the DNA at –30°C to –15°C.

DNA extraction using kits based on spin-column technology can be automated using the QIAcube®.

* Extraction kit allows simultaneous extraction of ASFV DNA and CSFV RNA.

# 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 material safety data sheets (MSDSs), 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
- 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 DNA. 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* ASFV 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 PCR for detection of African swine fever virus

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 (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.
- 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 DNA (Table 1).

   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 negative control (Negative Control) instead of sample DNA.

**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. Select the green and yellow channels on the Rotor-Gene Q.

   Important: Set a fixed gain of +4 in the green and +1 in the yellow 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 |
|---|---|---|
| ASFV | FAM | green |
| Internal Control | HEX/JOE™* | yellow |
| Passive reference† | ROX™ | |

* Use the option appropriate for your thermal cycler.

† Internal reference for use with Applied Biosystems® ABI PRISM® Sequence Detection Systems.

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

**Table 3. Real-time PCR Protocol for ASFV**

| Temperature | Time | Number of cycles |
|---|---|---|
| 95°C | 15 min | 1 |
| 95°C | 15 s | 40 |
| 60°C* | 60 s | |

* Fluorescence data collection. Approximate run time (Rotor-Gene Q): 96min.

5. Run the real-time RT-PCR protocol according to Table 4 if running the *virotype* CSFV assay simultaneously.

**Table 4. Real-time RT-PCR Protocol for parallel amplification of ASFV and CSFV†**

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

† Valid for *virotype* CSFV RT-PCR Kit only.
‡ Fluorescence data collection. Approximate run time (Rotor-Gene Q): 118 min

# 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 summarized in Table 5 on page 17.

The sample is positive for ASFV, 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 ASFV DNA 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 ASFV, 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 it is recommended that the respective individual samples are 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 DNA in nuclease free water, to repeat the DNA 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***

| | Reporter | |
|---|---|---|
| **Sample result** | **FAM (pathogen)** | **HEX (IC)** |
| ASFV positive | X | X |
| ASFV positive (strong positive) | X | |
| ASFV 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 15.

# 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, visit **www.qiagen.com**).

# Ordering Information

| Product | Contents | Cat. no. |
|---|---|---|
| *virotype* ASFV PCR Kit (24) | For 24 reactions: Master Mix, Positive Control, Negative Control | 281903 |
| *virotype* ASFV PCR Kit (96) | For 96 reactions: Master Mix, Positive Control, Negative Control | 281905 |
| **Related products** | | |
| *virotype* CSFV RT-PCR Kit (96)* | For 96 reactions: Master Mix, Positive Control, Negative Control | 281805 |
| *virotype* PEDV/TGEV RT-PCR Kit (96)* | For 96 reactions: Master Mix, Positive Control, Negative Control | 283605 |
| *virotype* BTV RT-PCR Kit (96)* | For 96 reactions: PCR Mix, Enzyme Mix, Positive Control, Negative Control | 280435 |
| *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 |

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

| Product | Contents | Cat. no. |
|---|---|---|
| *bactotype*® Mycoplasma Mg/Ms PCR Kit (96)* | For 96 reactions: Master Mix, Positive Control, Negative Control | 288105 |
| *bactotype* MAP PCR Kit (96)* | For 96 reactions: Master Mix, Internal Control DNA, Positive Control, Negative Control | 285905 |
| 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 |
| DNeasy Blood & Tissue Kit (50)* | For 50 preps: 50 DNeasy Mini Spin Columns, Proteinase K, Buffers, Collection Tubes (2 ml) | 69504 |
| QIAamp DNA Blood Mini Kit (50)* | For 50 DNA preps: 50 QIAamp Mini Spin Columns, QIAGEN Protease, Reagents, Buffers, Collection Tubes (2 ml) | 51104 |
| 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 |

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

QIAGEN offers 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 *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.

**Limited License Agreement for *virotype* ASFV PCR Kit**

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# Notes

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