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virotype® ASFV 2.0 PCR Kit Handbook

For detection of DNA from *African Swine Fever Virus* (ASFV)

Licensed in accordance with § 11 (2) of the German Animal Health Act
MA No.: FLI-C 079

REF 96 reactions (cat. no. VT281925)

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INDICAL BIOSCIENCE

Contents

Kit contents

virotype ASFV 2.0 PCR Kit **Cat. no.** **Number of reactions**	**(96)** **VT281925** **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
IC-DNA (tube with transparent cap)	1 x 200 µl
Handbook	1

Intended use

The virotype ASFV 2.0 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-C 079) 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 pigs and wildboar

Quality control

In accordance with INDICAL's ISO-certified Quality Management System, each lot of virotype ASFV 2.0 PCR Kit is tested against predetermined specifications to ensure consistent product quality.

Storage

The components of the virotype ASFV 2.0 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 ASFV 2.0 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 2.0 PCR Kit allows early detection of the pathogen in individual as well as in pooled samples of serum, plasma, EDTA-blood, tissue, and swab material from pigs and wild boar.

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 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 ASFV 2.0 PCR Kit contains all of the necessary reagents for the detection of ASFV DNA, including a Positive and Negative Control.

The kit contains two internal controls. The endogenous control (EC) detects a housekeeping gene present within the sample and the exogenous control (IC-DNA) permits tests for successful extraction and amplification by adding it to the DNA purification procedure.

Both internal control systems exclude the possibility of false-negative results.

The kit uses three specific primer/probe combinations:

- FAM™ fluorescence for DNA of ASFV
- HEX™ fluorescence for the endogenous Internal Control (β-actin present within the sample)
- Cy®5 fluorescence for the exogenous Internal Control (IC-DNA extracted during DNA purification)

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 2.0 PCR Kit can be used for the detection of ASFV DNA from serum, plasma, EDTA-blood, tissue, and swab samples from pigs and wild boars.

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.

Note: For use in Germany the specifications described in the „Amtliche Methodensammlung“ apply.

Prior to real-time PCR, viral DNA must be extracted from the starting material. The exogenous internal control DNA (IC-DNA) must be added to the **lysis buffer** prior to the extraction procedure. In most cases, 2 µl IC-DNA per sample is suitable.

INDICAL offers a range of validated kits for the extraction of DNA from animal samples.

Extraction based on magnetic beads:

- **IndiMag® Pathogen Kit *** (SP947457; formerly MagAttract 96 cador® Pathogen Kit)
- **IndiMag Pathogen Kit w/o plastics** (SP947257; formerly MagAttract 96 cador Pathogen Kit w/o Plastics)

Extraction based on spin columns:

- **IndiSpin® Pathogen Kit *** (SP54104, SP54106; formerly QIAamp® cador Pathogen Mini Kit)
- **IndiSpin QIAcube® HT Pathogen Kit** (SP54161; formerly cador Pathogen 96 QIAcube HT Kit) – not suitable for blood samples

* suitable for simultaneous extraction of ASFV DNA und CSFV RNA

Note: When using difficult sample material, it is recommended to use INDICAL’s „Pretreatment T4 (phenol extraction)“.

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

For further information on automated and manual extraction of ASFV DNA from different sample types, refer to the respective handbook or contact INDICAL Support at **support@indical.com**.

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 DNA. 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 ASFV 2.0 PCR Kit to test for successful amplification of the target.

Extraction and amplification control

For increased process safety and convenience, two extraction and amplification control assays are included in the test kit.

An **endogenous** Internal Control (EC) detects a housekeeping gene present within the sample, whereas the **exogenous** Internal Control detects IC-DNA, which must be added to the lysis buffer prior to extraction. The use of both control systems allows extraction and amplification to be monitored, as well as the sample quality.

It is strongly recommended to add the IC-DNA to the lysis buffer prior to extraction. This allows to monitor extraction and amplification also in samples that would show at least partial inhibition due to the sample quality.

Protocol: Real-time PCR for detection of DNA from *African Swine Fever Virus*

Important points before starting

- Please read „Important notes“ on page 10 before starting.
- It is strongly recommended to use the IC-DNA provided with the kit to monitor extraction and amplification, as well as any partial inhibition. Please add the respective volume to the lysis buffer prior to extraction.
- 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.

Table 2. Filter settings for the reporter

Pathogen/ internal control	Reporter
ASFV	FAM
Endogenous Internal Control (EC)	HEX/ JOE™[1]
Exogenous Internal Control (IC-DNA)	Cy5
Passive reference[2]	ROX™

1 Use the option appropriate for your thermal cycler.
2 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 2.0 PCR Kit.

Table 3. Real-time PCR protocol for ASFV 2.0

Step	Temperature	Time	Number of cycles
Initial Activation	95°C	2 min	1
2-step cycling			
Denaturation	95°C	5 s	40
Annealing/Extension*	60°C	30 s	

* Fluorescence data collection. Approximate run time 60 min (CFX96, Bio-Rad™)

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 simultaneous amplification of ASFV 2.0 and CSFV[1]

Step	Temperature	Time	Number of cycles
Reverse Transcription	45°C	10 min	1
Initial Activation	95°C	10 min	1
3-step cycling			
Denaturation	95°C	15 s	
Annealing*	57°C	30 s	40
Extension	72°C	35 s	

1 valid for viotype CSFV RT-PCR Kit only.

* Fluorescence data collection. Approximate run time 118 min (Rotor-Gene Q)

Data analysis and interpretation

Interpretation of results

For the assay to be valid the Positive Control must give a signal in the FAM, HEX and Cy5 channels with a C_T[1] < 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 18.

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

- The sample yields a signal in the FAM channel (regardless of any signal in the HEX and/ or Cy5 channel).
- The Positive Control yields a signal in all channels (FAM, HEX and Cy5).
- The Negative Control does not yield a signal in the FAM, HEX and Cy5 channel.

Note that very high concentrations of ASFV DNA in the sample may lead to a reduced or no signal of internal controls due to competition.

[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 negative for ASFV, and the assay is valid, if the following criteria are met:

- The sample does not yield any signal in the FAM channel.
- The sample yields a signal in only the HEX channel if no IC-DNA was used or in the HEX and Cy5 channel if IC-DNA was used.
- The Positive Control yields a signal in all channels (FAM, HEX and Cy5).
- The Negative Control does not yield a signal in the FAM, HEX and Cy5 channel.

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

- The sample yields no signal in the FAM, HEX and Cy5 channel.

If no signal is detected in the FAM (ASFV), the HEX (endogenous Internal Control, EC) and Cy5 (exogenous Internal Control, IC-DNA) channel, the result is inconclusive. The absence of a signal for the housekeeping gene and the IC-DNA indicates strong PCR inhibition and/or other malfunctions, e.g. during extraction.

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.

Additional information given by the endogenous and exogenous Internal Control systems:

The **lack of Cy5 fluorescence signal** can be caused by insufficient sample extraction, competition with a strong positive ASFV signal, PCR inhibition or will occur in cases where the IC-DNA had not been added. Higher C_T-values in the Cy5 channel of a sample compared to the majority of samples may indicate partial inhibition in the sample.

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.

The **lack of HEX fluorescence signal** in presence of a signal in the Cy5 channel indicates poor sample quality and/ or sample amount.

Table 5. Results interpretation table*

Sample result	FAM (ASFV)	HEX (EC)	Cy5 (IC-DNA)	Interpretation
ASFV positive	X	X	X	Valid
ASFV positive	X	X		Valid (extraction without IC-DNA)
ASFV strong positive	X	(X)	(X)	Valid (no EC and/ or IC-DNA signal due to competition)
ASFV negative		X	X	Valid
Poor sample quality			X	Successful extraction (no EC-signal due to poor sample quality or amount; recommendation to test a new sample; also applies to non-animal samples
Partial PCR inhibition		X	(X)	No or weak C_T value for IC-DNA when added to the extraction (recommendation to test 1:5 dilution of the sample)
Inconclusive				Not valid (no signal for both EC and IC-DNA possibly due to failure during extraction or PCR)

* 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 and Cy5 channel. The Negative Control must yield no signal in any 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-2526-EN-002	Jan 2020	Adjustment MA number
HB-2526-EN-001	Nov 2019	Product launch

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