May 2025 | EN

virotype® ASFV 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-B 670

REF 96 reactions (cat. no. VT281905)

INDICAL BIOSCIENCE GmbH, Deutscher Platz 5b,
04103 Leipzig, Germany

INDICAL BIOSCIENCE

Contents

Kit contents

virotype ASFV PCR Kit	(96)
Cat. no.	VT281905
Number of reactions	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)	1 x 150 µl
Handbook	1

Intended use

The virotype ASFV PCR Kit is intended for the detection of DNA from *African Swine Fever Virus* (ASFV) in blood, tissue, swabs, and samples in stabilizing transport media 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

 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 PCR Kit is tested against predetermined specifications to ensure consistent product quality.

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 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 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 (WOAH).

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 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 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:

- FAM™ fluorescence for DNA of ASFV
- HEX™ fluorescence for the endogenous internal control (EC, β-actin present within the sample)

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 pig 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.

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

Extraction based on magnetic beads:

- IndiMag® Pathogen Kit (SP947457)
- IndiMag Pathogen Kit w/o plastics (SP947257)
- IndiMag Pathogen IM2 Cartridge (SP957654C608)
- IndiMag Pathogen IM48 Cartridge (SP947654P608, SP947654P224)
- IndiMag Pathogen KF96 Cartridge (SP947855P196, SP947855P496)

Extraction based on spin columns:

- IndiSpin® Pathogen Kit * (SP54104, SP54106)
- IndiSpin QIAcube® HT Pathogen Kit (SP54161 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 „Pretreatment T4 (phenol extraction)".

If real-time PCR is not performed immediately after extraction, store the DNA at -20°C or at -80°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 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 DNA from *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.

   Note: 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 Csontrol	Reporter
ASFV	FAM
Internal Control	HEX/ JOE™[1]
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.

Table 3. Real-time PCR protocol for ASFV

Step	Temperature	Time	Number of cycles
Initial Activation	95°C	15 min	1
2-step cycling			
Denaturation	95°C	15 s	40
Annealing/Extension*	60°C	60 s	

* Fluorescence data collection. Approximate run time 100 min (CFX96, BioRad)

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[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 4 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 the HEX channels.
- The Positive Control yields a signal in both the FAM and the 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.

[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 yields a signal in only the HEX 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 channels.

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 4. Results interpretation table*

Sample result	FAM (ASFV)	HEX (IC)
ASFV positive	X	X
ASFV positive (strong positive)	X	
ASFV negative		X
inconclusive		

* 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.

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 afosa, bactotype, cador, cattletype, flocktype, pigtype, Svanovir and virotype products.

For up-to-date licensing information and product-specific disclaimers, see the respective INDICAL kit handbook or user manual.

Notes

Limited License Agreement for virotype ASFV 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. INDICAL 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.indical.com. Some of these additional protocols have been provided by INDICAL users for INDICAL users. These protocols have not been thoroughly tested or optimized by INDICAL. INDICAL neither guarantees them nor warrants that they do not infringe the rights of third-parties.
2. Other than expressly stated licenses, INDICAL makes no warranty that this kit and/or its use(s) do not infringe the rights of third-parties.
3. This kit and its components are licensed for one-time use and may not be reused, refurbished, or resold.
4. INDICAL specifically disclaims any other licenses, expressed or implied other than those expressly stated.
5. The purchaser and user of the kit agree not to take or permit anyone else to take any steps that could lead to or facilitate any acts prohibited above. INDICAL may enforce the prohibitions of this Limited License Agreement in any Court, and shall recover all its investigative and Court costs, including attorney fees, in any action to enforce this Limited License Agreement or any of its intellectual property rights relating to the kit and/or its components.

For updated license terms, see www.indical.com.

Change index

Handbook	Version	Change
HB-1881-EN-006	May 2025	Deletion of harmonized PCR protocol due to discontinuation of virotype CSFV RT-PCR Kit; editorial changes
HB-1881-EN-005	Feb 2021	Adjustment Intended Use and implementation of new extraction kits

INDICAL BIOSCIENCE

Ordering: **www.indical.com/contact**
Technical Support: **support@indical.com**
Website: **www.indical.com**