VetMAX™ African Swine Fever Virus Detection Kit

TaqMan® real-time PCR detection of African swine fever virus

Catalog Number A28809

Doc. Part No. 100027918 Pub. No. MAN0010783 Rev. C.0

Technology	Species	Samples	Test type
Real-time PCR (DNA) • Duplex assay • Exogenous IPC	Swine	Blood Serum	Individual Pooled samples (5 or 10 samples)
		Tissues	Individual

WARNING! Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves. Safety Data Sheets (SDSs) are available from **thermofisher.com/support**.

Product description

The Applied Biosystems™ VetMAX™ African Swine Fever Virus Detection Kit (Cat. No. A28809) enables detection of the African swine fever virus (ASFV) in swine blood, serum, or tissues by real-time PCR amplification of the ASFV P72 gene.

The assay is a single-well real-time PCR in which ASFV and exogenous Internal Positive Control (IPC) targets are amplified and detected using fluorescent TaqMan® probes.

The kit includes:

- 3 - Mix ASFV: Contains primers, TaqMan® probes, buffer, and enzyme for optimized duplex real-time PCR amplification of ASFV and IPC targets.
- 4a - EPC ASFV: Nucleic acid template for P72 target amplification. It serves as an external positive control for the real-time PCR reaction, and it is used to set the cycle threshold (C_t) for evaluating test results.
- 5 - IPC ASFV: Internal positive control added to each sample and control at the lysis step of the DNA extraction procedure. It serves as a control for the DNA purification process, and it is used to monitor for the presence of PCR inhibitors.

Contents and storage

Component	Amount[1]	Storage[2]
3 – Mix ASFV	2 × 1000 µL	–30°C to –10°C
4a – EPC ASFV	2 × 90 µL	
5 – IPC ASFV	1 × 500 µL	

[1] Sufficient for 100 25-µL real-time PCR reactions.

[2] See packaging for expiration date.

Required materials not supplied

Unless otherwise indicated, all materials are available through **thermofisher.com**. MLS: Fisher Scientific (**fisherscientific.com**) or other major laboratory supplier.

Item	Source
Applied Biosystems™ 7500 Real-Time PCR System	Contact your local sales office.
96-well plate, strip tubes (8- or 12-wells), microtubes or capillaries compatible with thermal cycler used	MLS
Nuclease-free pipettes and filtered pipette tips	MLS
Two ice buckets or refrigerated racks: • One for the PCR setup area where the PCR master mix is prepared • One for the area where DNA samples and controls are prepared	MLS
Plate covers or caps compatible with the plates, strip tubes, microtubes, or capillaries	MLS
Nuclease-free reagent tubes for preparing master mix	MLS
Nuclease-free Water (not DEPC-Treated)	AM9939
1X TE Buffer	MLS

Procedural guidelines

- For each real-time PCR run, include the controls indicated in “Set up the PCR reactions” on page 2.
- Follow “Good laboratory practices for PCR and RT-PCR” on page 4 to prevent false positives and contamination of test samples with PCR products.

Requirements for input DNA

We recommend using the MagMAX™ Pathogen RNA/DNA Kit (Cat. No. 4462359) for DNA extraction from biological samples, but you can also use other high quality DNA extraction methods after proper validation in your laboratory. In addition, prepare mock-purified sample using nuclease-free water as the starting material and the same DNA isolation method used for test samples.

IMPORTANT! Add 5 µL of 5 - IPC ASFV to the lysis solution used for DNA isolation for each sample and extraction control.

Before you begin

1. Thaw reagents and samples:
   a. Thaw 3 – Mix ASFV in an ice bucket or refrigerated rack.
   b. Thaw 4a – EPC ASFV, 5 – IPC ASFV, and DNA samples in a separate ice bucket or refrigerated rack.
2. Thoroughly mix the contents of each tube by vortexing, then briefly centrifuge.

Store thawed reagents, controls, and samples at 2–8°C until use.

Set up the PCR reactions

1. Dispense 20 µL of 3 – Mix ASFV to the appropriate number of PCR plate wells, strip tubes, or capillaries.
2. Add sample or control according to the following table:

Sample type	Component	Volume per reaction
Test sample	Sample DNA	5.0 µL
Positive control	4a – EPC ASFV	5.0 µL
Extraction control	Mock-purified sample	5.0 µL
No-template control (NTC)	Nuclease-free Water	5.0 µL

3. Seal each plate or tube, mix, then centrifuge briefly to bring the contents to the bottom of the plate wells or tubes.

Set up and run the real-time PCR instrument

1. Following the manufacturer's instructions, set up the real-time PCR run using the following parameters.
   - Reaction volume: 25 µL
   - Passive reference: ROX™ dye (included in 3 – Mix ASFV)

     Note: ROX™ dye must be set up if the instrument is capable of detecting it. Real-time PCR instruments that do not detect ROX™ dye can be used without affecting the accuracy of the reading.
   - Select detectors and assign TaqMan® probe reporter dyes and quenchers for each well, tube, or capillary used in the analysis.

Target	Reporter	Quencher
ASFV	FAM™ dye	Non-fluorescent quencher (NFQ)
IPC	VIC™ dye	TAMRA™ dye[1]

[1] TAMRA™ dye must be set up for real-time PCR analysis if the instrument is capable of detecting it. Real-time PCR instruments that do not detect TAMRA™ dye can be used without affecting the accuracy of the reading.

   - Thermal cycling program:

Stage	Repetitions	Temperature	Time
1	1	50°C	2 minutes
2	1	95°C	10 minutes
3	45	95°C	15 seconds
		60°C	1 minute

2. Run the thermal cycler program, collecting real-time amplification data during stage 3.

Guidelines for data analysis

- Follow the instrument user guide for raw data analysis.
- Set the thresholds for each target separately.
- Interpret the results for each control and sample according to the obtained C_t values as indicated in the following sections.

Validation criteria

Refer to the C_{tQC} values in the Certificate of Analysis for the manufacturing lot of the kit. The test is validated if the following criteria are met:

Reaction type	ASFV target (FAM™ dye)	IPC target (VIC™ dye)	Interpretation
Positive control	$C_t = C_{tQC}$ ASFV ± 3 C_t[1]	$C_t < 45$ or $C_t > 45$[2]	PCR is validated.
Extraction control[3]	$C_t > 45$	$C_t = C_{tQC}$ IPC ± 3 C_t[4]	DNA extraction is validated.
No-template control	$C_t > 45$	$C_t > 45$	PCR reagents are validated.

[1] See the EPC table in the Certificate of Analysis.
[2] The IPC value of the positive control is not used for test validation.
[3] Use the extraction control prepared using the same extraction procedure as the samples.
[4] See the IPC table in the Certificate of Analysis.

Interpretation of results

ASFV target (FAM™ dye)	IPC target (VIC™ dye)	Interpretation
$C_t < 45$	$C_t < 45$ or $C_t > 45$	ASFV is detected.
$C_t > 45$	$C_t = C_t$ of extraction control ± 3 C_t [1]	ASFV is not detected.
$C_t > 45$	C_t is outside this range: C_t of extraction control ± 3 C_t[1]	Invalid result.[2]

[1] The C_t value of the extraction control must first be validated as described in “Validation criteria” on page 3.
[2] The result is invalid due to a non-compliant IPC result.

Retest samples with invalid results

1. Dilute the DNA samples 1:10 in 1X TE buffer.
2. Repeat the real-time PCR procedure with 5 µL of the diluted DNA, then interpret the results as follows.

Result	Interpretation
The diluted DNA is positive for ASFV.	The result is validated.
The diluted DNA is negative for ASFV, and the IPC result is compliant.	
The diluted DNA is negative for ASFV, but the IPC result is non-compliant.	The result is invalid.

3. For diluted samples with invalid results, repeat the DNA isolation procedure on a new aliquot of the original sample lysate, then repeat the test.

Good laboratory practices for PCR and RT-PCR

When preparing samples for PCR or RT-PCR amplification:

- Wear clean gloves and a clean lab coat.
  - Do not wear the same gloves and lab coat that you have previously used when handling amplified products or preparing samples.
- Change gloves if you suspect that they are contaminated.
- Maintain separate areas and dedicated equipment and supplies for:
  - Sample preparation and reaction setup.
  - Amplification and analysis of products.
- Do not bring amplified products into the reaction setup area.
- Open and close all sample tubes carefully. Avoid splashing or spraying samples.
- Keep reactions and components capped as much as possible.
- Use a positive-displacement pipettor or aerosol-resistant barrier pipette tips.
- Clean lab benches and equipment periodically with 10% bleach solution or DNA decontamination solution.

World Organisation for Animal Health (OIE) Certification

Validated and certified by the OIE as fit for the purposes defined in the kit insert. Registration number: 20200114.

Customer and technical support

Visit **thermofisher.com/support** for the latest in services and support, including:

- Worldwide contact telephone numbers
- Product support, including:
  - Product FAQs
  - Software, patches, and updates
  - Training for many applications and instruments
- Order and web support
- Product documentation, including:
  - User guides, manuals, and protocols
  - Certificates of Analysis
  - Safety Data Sheets (SDSs; also known as MSDSs)

    Note: For SDSs for reagents and chemicals from other manufacturers, contact the manufacturer.

Limited product warranty

Life Technologies Corporation and/or its affiliate(s) warrant their products as set forth in the Life Technologies' General Terms and Conditions of Sale found on Life Technologies' website at **www.thermofisher.com/us/en/home/global/terms-and-conditions.html**. If you have any questions, please contact Life Technologies at **www.thermofisher.com/support**.

Manufacturer: Laboratoire Service International (LSI) | 6 Allée des Ecureuils – Parc Tertiaire de Bois-Dieu | 69380 Lissieu – France

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Revision history: Pub. No. MAN0010783 (English)

Revision	Date	Description
C.0	21 July 2020	Added the World Organisation for Animal Health (OIE) logo and registration number.
B.0	8 February 2018	• Updated to the current document template, with associated updates to the warranty, trademarks, and logos. • Minor edits to align with current style.
A.0	2 April 2015	Baseline for revision history.