

VetMAX™ African Swine Fever Virus Detection Kit 2.0

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

Catalog Number A57008

Doc. Part No. 100119745 Pub. No. MAN0028501 Rev. C

Technology	Species	Samples ^[1]	Test type
Real-time PCR (DNA) <ul style="list-style-type: none"> • Triplex assay • (Optional) Exogenous IPC • Endogenous IPC 	Porcine and wild boar	Whole blood, serum, tissues, lymphoid organs, oral fluids, meat exudate, swabs, blotting paper, wipes (environmental samples)	Individual Pooled samples (up to 20 samples ^[2])

^[1] For France, only the sample types indicated in the French specifications document ANSES (2024) for presentation of PCR reagents that detect African Swine Fever Virus (ASFV) have been validated.

^[2] Pooling recommendations and regulations may vary by country. For Poland, please follow Polish requirements: pool up to 5 samples. For France, please follow French requirements: pool up to 5 whole blood samples.



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](https://www.thermofisher.com/support).

Product description

The Applied Biosystems™ VetMAX™ African Swine Fever Virus Detection Kit 2.0 (Cat. No. [A57008](#)) enables detection of the African swine fever virus (ASFV) in samples (whole blood, serum, tissues, organs, oral fluids, meat exudate, swabs, blotting paper, and wipes) by real-time PCR amplification. The assay is a single-well real-time PCR in which DNA targets are amplified and detected using fluorescent TaqMan™ probes. The targets are ASFV and two forms of Internal Positive Control (IPC): exogenous and endogenous.

The kit includes the following components:

- 3 – Mix ASFV 2.0 – Contains primers, TaqMan™ probes, buffer, and enzyme for optimized triplex real-time PCR amplification of ASFV and IPC targets.
- 4a – EPC ASFV 2.0 – Nucleic acid template for 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 2.0 – Exogenous internal positive control that can be added to each sample and control of the DNA extraction procedure. It serves as a control for the DNA extraction process, and it is used to monitor for the presence of PCR inhibitors.
- 6 – Negative Control ASFV 2.0 – Nuclease-free water that serves as an external negative control for the real-time PCR reactions.

Contents and storage

Table 1 VetMAX™ African Swine Fever Virus Detection Kit 2.0 (Cat. No. [A57008](#))

Component	Cap color	Amount ^[1]	Storage ^[2]
3 – Mix ASFV 2.0	Green cap	2 × 500 µL	–30°C to –10°C
4a – EPC ASFV 2.0	Brown cap	2 × 90 µL	
5 – IPC ASFV 2.0	Yellow cap	1 × 550 µL	
6 – Negative Control ASFV 2.0	White cap	1 × 180 µL	

^[1] Sufficient for 100 15-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](https://www.thermofisher.com). "MLS" indicates that the material is available from [fisherscientific.com](https://www.fisherscientific.com) or another major laboratory supplier. Catalog numbers that appear as links open the web pages for those products.

Item	Source
Real-time PCR instrument, one of the following:	
Applied Biosystems™ 7500 Real-Time PCR System <ul style="list-style-type: none"> • Precision Plate Holder for 7500 Real-Time PCR Systems (Cat. No. A24820) • Precision Plate Holder for 0.2 mL tubes and strips (Cat. No. 4367033) 	Contact your local sales office.
Applied Biosystems™ 7500 Fast Real-Time PCR System <ul style="list-style-type: none"> • Precision Plate Holder for 7500 Fast Real-Time PCR Systems (Cat. No. 4359652) • 7500 Fast Precision Plate Holder for 0.1 mL tube strips (Cat. No. A29252) 	
QuantStudio™ 3 Real-Time PCR System	
QuantStudio™ 5 Real-Time PCR System	
QuantStudio™ 7 Pro Real-Time PCR Instrument	

Item	Source
Equipment	
Nuclease-free pipettors	MLS
Two ice buckets or refrigerated racks: <ul style="list-style-type: none"> • 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
Tubes, plates, and other consumables	
MicroAmp™ Fast Optical 96-Well Reaction Plate, 0.1 mL	4346907
MicroAmp™ Optical 96-Well Reaction Plate, 0.2 mL	4316813
MicroAmp™ Optical Adhesive Film	4311971
MicroAmp™ Optical 8-Tube Strip, 0.2 mL	4316567
MicroAmp™ Optical 8-Cap Strips	4323032
MicroAmp™ 96-Well Support Base	4379590
Reagents	
1X Tris-EDTA (TE) buffer	MLS
Other consumables	
Nuclease-free reagent tubes for preparing the reaction mixes	MLS
Aerosol-resistant pipette tips	MLS

Procedural guidelines

- Strictly follow all national safety regulations for ASFV.
- Materials from another laboratory supplier can be used.
- 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™ CORE Nucleic Acid Purification Kit (Cat. Nos. [A32700](#) and [A32702](#)) for DNA extraction from biological samples; you can also use other high-quality DNA extraction methods. In addition, prepare the mock sample using nuclease-free water as the starting material and the same DNA extraction method used for test samples.

IMPORTANT! If an exogenous IPC is necessary for your experiment, add 5 µL of the 5 – IPC ASFV 2.0 to each sample and extraction control during DNA extraction.

Before you begin

1. Thaw reagents and samples:
 - a. Thaw the 3 – Mix ASFV 2.0 in an ice bucket or refrigerated rack.
 - b. Thaw the following components in a separate ice bucket or refrigerated rack.
 - 4a – EPC ASFV 2.0
 - 5 – IPC ASFV 2.0–Mandatory for wipes (environmental samples), and optional for other sample types.
 - 6 – Negative Control ASFV 2.0
 - DNA samples
 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

IMPORTANT! Dispense the 3 – Mix ASFV 2.0 at room temperature. Use a MicroAmp™ 96-Well Support Base.

1. Dispense 10 µL of the 3 – Mix ASFV 2.0 to the appropriate number of PCR plate wells, strip tubes, or capillaries.
2. Add sample or control according to the following table:

Reaction type	Component	Volume per reaction
Test sample	Sample DNA	5.0 µL
Positive control	4a – EPC ASFV 2.0	5.0 µL
(Optional) Exogenous Internal Positive Control (IPC) ^[1]	5 – IPC ASFV 2.0 diluted at a 1:10 ratio	1.0 µL
Extraction control	Mock sample	5.0 µL
No-template control (NTC)	6 – Negative Control ASFV 2.0	5.0 µL

^[1] If IPC has not been added during the sample extraction, it can be added to 3 – Mix ASFV 2.0 during the “Set up the PCR reactions”.

3. Seal each plate or tube, mix, then centrifuge briefly to collect the contents.

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: 15 µL
 - Passive reference: ROX™ dye (included in the 3 – Mix ASFV 2.0)

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.

- Thermal cycler program: Usable in fast mode or standard mode (QuantStudio™ 5 Real-Time PCR System)

Stage	Repetitions	Temperature	Time
1	1	95°C	5 minutes (05'00")
2	40	95°C	5 seconds (00'05")
		60°C ^[1]	20 seconds (00'20")

^[1] Collection of fluorescence data during the 60°C – 20 seconds stage.

- Thermal cycler program: Usable in fast mode or standard mode (7500/7500 Fast Real-Time PCR System)

Stage	Repetitions	Temperature	Time
1	1	95°C	5 minutes (05'00")
2	40	95°C	5 seconds (00'05")
		60°C ^[1]	31 seconds (00'31")

^[1] Collection of fluorescence data during the 60°C – 31 seconds stage.

- Select or create dye detectors, then assign to each well or tube.

Target	Reporter	Quencher
ASFV	FAM™ dye	Non-fluorescent quencher (NFQ)
Exogenous IPC ^[1]	Cy5™ dye	
Endogenous IPC	VIC™ dye	

^[1] Included in the kit as component 5 – IPC ASFV 2.0.

- Run the appropriate thermal cycler program, collecting real-time amplification data during the 60°C incubation.

Guidelines for data analysis

- Follow the instrument user guide for raw data analysis.
- Set the thresholds for each target separately.
- See the Certificate of Analysis for the manufacturing batch of the kit to validate the run and interpret the results.

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)	(Optional) Exogenous IPC target (Cy5™ dye)	Endogenous IPC target (VIC™ dye)	Interpretation
Positive control	$C_t = C_{tQC} \text{ ASFV} \pm 3 C_t^{[1]}$	$C_t > 40^{[2]}$	$C_t > 40^{[2]}$	PCR is validated.
Extraction control ^[3]	$C_t > 40$	$C_t = C_{tQC} \text{ IPC} \pm 3 C_t^{[4]}$	$C_t > 40^{[5]}$	DNA extraction is validated.
No-template control	$C_t > 40$	$C_t > 40$	$C_t > 40$	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 that was prepared using the same extraction procedure as the samples.

^[4] See the IPC table in the Certificate of Analysis.

^[5] The IPC value of the extraction control is not used for test validation.

Interpretation of results

Table 2 Interpretation of the results if only an endogenous IPC target was used in the experiment

ASFV target (FAM™ dye)	Endogenous IPC target (VIC™ dye)	Interpretation
$C_t < 40$	Any value	ASFV is detected.
$C_t > 40$	$C_t < 40$	ASFV is not detected.
$C_t > 40$	$C_t > 40$	Invalid result. ^[1]

^[1] The result is invalid due to experimental failure.

Table 3 Interpretation of the results if dual IPC targets were used in the experiment

ASFV target (FAM™ dye)	Exogenous IPC target (Cy5™ dye)	Endogenous IPC target (VIC™ dye)	Interpretation
$C_t < 40$	Any value ^[1]	Any value	ASFV is detected.
$C_t > 40$	$C_t = C_t$ of extraction control $\pm 3 C_t^{[2]}$	$C_t < 40$ For wipes: Any value	ASFV is not detected.
$C_t > 40$	C_t is outside of this range: C_t of extraction control $+ 3 C_t^{[2]}$	Any value	Invalid result. ^[3]
$C_t > 40$	$C_t = C_t$ of extraction control $\pm 3 C_t^{[2]}$	$C_t > 40$	Invalid result. ^[4]

^[1] A strong positive result for ASFV may compete with the exogenous IPC target.

^[2] The C_t value of the extraction control must first be validated as described in "Validation criteria" on page 3.

^[3] The result is invalid due to inhibition, or a non-compliant IPC result.

^[4] The result is invalid due to failure during the experiment in the case of cellular samples (wipes excluded).

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 extraction procedure on a new aliquot of the original sample lysate, then repeat the test.

Good laboratory practices for PCR and RT-PCR

- 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.

Customer and technical support

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 - 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

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For descriptions of symbols on product labels or product documents, go to [thermofisher.com/symbols-definition](https://www.thermofisher.com/symbols-definition).

Revision history: Pub. No. MAN0028501 C

Revision	Date	Description
C	6 January 2025	New sample types (swabs, blotting paper, and wipes) were validated and added to satisfy ANSES requirements for this kit.
B.0	27 June 2023	A statement was added regarding pooled sample requirements.
A.0	15 December 2022	New document for VetMAX™ African Swine Fever Virus Detection Kit 2.0.

The information in this guide is subject to change without notice.

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