



ASFV FAST TIME

Reference: ADI551-100

Test for the detection of African Swine Fever Virus (ASFV) by real time enzymatic amplification
PCR Test – 100 reactions

For veterinary *in vitro* use only



Sample	Individual analysis	Pool of sample possible*, up to:
EDTA Blood, serum, plasma or cell culture supernatant	✓	20
Tissue (spleen, tonsil, ganglion...)	✓	10
swab from blood or exudate	✓	✗
Bone marrow	✓	✗

* Depending on the epidemiological case and on the quality of samples.

Kit composition

Content		ADI551 Kit 100 reactions
A5	Amplification solution	2 x 1000 µL tubes with green cap (Ready to use)
ASFV CTL+	ASFV positive control	1 tube with purple cap (To reconstitute)
NF-Water	Nuclease-Free Water	1 x 1000 µL tube with white cap (Ready to use)

Revision history

Date	Version	Modifications
05/2019	NE551-02	Last version
09/2022	V01	Switch to simplified format Remove of 50 reactions reference Addition of plasma and bone marrow samples

Note: minor typographical, grammar and formatting changes are not included in the revision history.

A. Introduction

African swine fever is a highly contagious hemorrhagic disease that affects pigs, warthogs, wild boars from Europe and America. All age groups are susceptible to the disease. The pathogen responsible for ASF is a double-stranded DNA virus of the Asfarviridae family. The virus is transmitted among suidae through direct contact or by ingestion of contaminated meat products. Soft ticks of the genus *Ornithodoros* can also transmit the virus but are not obligatory vectors. In its highly virulent forms, African swine fever is characterized by high fever, loss of appetite, hemorrhages in the skin and internal organs; death occurs in 2 to 10 days on average. Mortality can reach 100 %.

Clinically, it is difficult to differentiate infections caused by classical swine fever virus from those due to the ASFV virus, hence the need for a differential diagnosis in the laboratory is requested. PCR is a highly sensitive and rapid tool for ASFV detection and differentiation from CSFV.

B. Test principle

ADIAVET™ ASFV FAST TIME test is based on gene amplification of ASFV specific DNA fragments. This test is intended to detect simultaneously, in one well:

- ASFV (FAM labelled probe)
- RNase P, an internal control of extraction and amplification specific from an endogenous nucleic acid (HEX labelled probe or its equivalent).

C. Storage conditions

On receipt, the kit should be stored at <-15 °C until the expiration date.

It is recommended to make fractions of A5 solution if it should be defrosted more than 3 times.

Do not thaw more than 3 times.

Store away from sunlight.

Do not mix reagents of two different batches.

D. Material required but not provided

- Real-time Thermal cycler and device.
- Instrument for homogenous mixing of tubes.
- Pipettes of 1 - 10 µL, 20 - 200 µL and 200 - 1000 µL.
- Nuclease-free filtered pipette tips.
- Nuclease-free microtubes of 1,5 mL and 2 mL.
- Powdered-free latex or nitrile gloves.
- Nuclease-free water.
- Kit for nucleic acids extraction.

Additional kits for method adoption and PCR

■ **Extraction Positive Control ASFV (Ref.: ADC55EPC).** Supplier reference material for method adoption that can also be used as a sentinel.

■ **LD_{PCR} Positive Control – ASFV (Ref.: ADC55LD)**
Confirmation of performances – LOD_{PCR} of ADIAVET™ ASFV FAST TIME kit.

E. Warnings and precautions

- For veterinary *in vitro* use only.
- For animal use only.
- For professional use only.
- All instructions must be read before performing the test and strictly respected.
- Do not use reagents after the expiration date.
- Do not use reagents if the packaging is damaged.
- Do not open PCR wells or tubes after amplification.
- Do not mix reagents from different batches.

- Do not open the PCR tubes after amplification.
- Used material must be disposed of in compliance with the legislation in force regarding environmental protection and biological waste management.
- This kit contains products of animal origin. Certified knowledge of the origin and/or sanitary state of the animals does not totally guarantee the absence of transmissible pathogenic agents. It is therefore recommended that these products be treated as potentially infectious and handled observing the usual safety precautions (do not ingest or inhale).

F. Nucleic acids extraction

1. Extraction kits

Nucleic acids must be extracted from the samples before using the kit. The RNA/DNA purification kits listed below are recommended by Bio-X Diagnostics:

Product name	Extraction system	Number of tests and reference
ADIAMAG	Magnetic beads	200 tests : ref. NADI003 800 tests : ref. NADI003-XL
ADIAMAG LB3 buffer	Buffer for magnetic beads for tissues	125 mL : ref. NADI004

For the extraction, consult the user manual version, available on the website, indicated on the certificate of analysis included in the PCR kit of interest.

Other purification kits can be used if they have been validated by the user.

After extraction, nucleic acid extracts can be kept on ice or at +2/8 °C for a few hours before use. For long term storage, they must be kept at a temperature below -15 °C.

2. Controls

Using controls allow to verify the reliability of the results. Controls can be included by series of analysis according to the recommendations defined by the standards in force (Cf. AFNOR U47-600...).

Controls	Validation of	Usage
No Template Control (NTC)	Absence of amplification contamination	5 µL NF-Water in a well per run
ASFV CTL+	ASFV target amplification	5 µL CTL+ in a well per run
Negative extraction control	Absence of contamination for the extraction and amplification	1 extraction (water or lysis buffer) per run
Positive extraction control	Extraction and amplification	1 extraction (Positive sample between 1 et 100X LOD _{Method}) per run

G. Procedure

1. Use of CTL+

Add **200 µL** of « **NF-Water** » per tube.

Homogenize the tubes using a mixer, such as vortex, > 20 seconds and until dissolution of the blue pellet.

After reconstitution, aliquot and store the solution at a temperature below -15 °C until the kit expiration date. Do not thaw more than 3 times.

For each assay, use **5 µL** of CTL+ in one of the dedicated wells (see § « Amplification », Step 2).

2. Amplification

Warning:

- Before starting, rehydrate or thaw reagents at room temperature in the dark.
- Homogenize all reagents and samples before use.
- Store reagents at a temperature below -15 °C after use.

Step 1: Dispense **20 µL** of amplification solution (A5) per well.

Step 2: Dispense **5 µL** of nucleic acids extracts and **5 µL** of controls in each dedicated well.

Use NF-Water for the No Template Control (NTC).

Step 3: Cover the wells with an appropriate optical film or caps.

Step 4: Start the PCR analysis.

The following programs are defined for ABI Prism thermocyclers (like 7500, QuantiStudio5, Step-one...) from Applied Biosystems, for Mx3000, Mx3005P and AriaMx from Agilent, for LightCycler from Roche Diagnostics, for Rotor-Gene Q from Qiagen, for CFX96 and Chromo 4 from Biorad and for MIC from BioMolecular System.

RNA FAST program*		DNA FAST program	
2 min. 50 °C		2 min. 95 °C	
10 min. 95 °C			
5 sec. 95 °C	45 cycles	5 sec. 95 °C	45 cycles
30 sec. 60 °C**		30 sec. 60 °C**	

*Compatible with ADIAVET™ CSFV REAL TIME (Bio-X Diagnostics, ref. ADI223)

** Reading and parameters for fluorescence acquisition:

Fluorochrome	Absorbance (nm)	Emission (nm)
FAM	494	520
HEX or equivalent	530	549
ROX	575	602

Note: The Quencher is non-fluorescent. The solution contains a passive reference read in the same spectra as ROX for ABI machines.

For other thermal cycler instruments, please contact your sales representative or the customer relations department.

H. Interpretation of results

Display all curves and position the threshold line for each fluorochrome.

1. Test validation

Amplification is valid if the following results are obtained.

Expected Ct (Threshold Cycle) values for the CTL+ are indicated on the certificate of analysis of the kit.

Controls	Amplification		Validation of
	FAM	HEX or equivalent	
No Template Control (NTC)	No	No	Absence of amplification contamination
ASFV CTL+	Yes	Yes	Target amplification
Extraction negative control	No	No	Absence of extraction contamination
Extraction positive control	Yes	Yes	Extraction and amplification steps

2. Results interpretation

Nucleic acids extraction and amplification are **valid** for each sample if at least one typical amplification curve is observed in FAM and/or HEX or equivalent.

Amplification		Interpretation
FAM	HEX or equivalent	ASFV
No	Yes	Undetected
Yes	Yes	Detected
Yes	No	Detected
No	No	Undetermined

« **Undetermined** »: no characteristic amplification curve.

Possible causes:

Defective PCR due to inhibitors, set up error, absence of samples, degraded samples and/or issue with nucleic acids extraction (loss or destruction of nucleic acids).

Recommendations:

Set up a new PCR assay using pure nucleic acids extracts and 10x dilutions in Nuclease-free water.

If the assay is inconclusive, perform a new nucleic acids extraction.

Symbols

Symbols	Signification
	Catalog number
	Manufacturer
	Temperature limitation
	Use by
	Batch code
	Consult Instructions for Use
	Contain sufficient for "n" tests
	For veterinary <i>in vitro</i> use only – For animal use only
	Keep away from sunlight

1 | Extract nucleic acids with

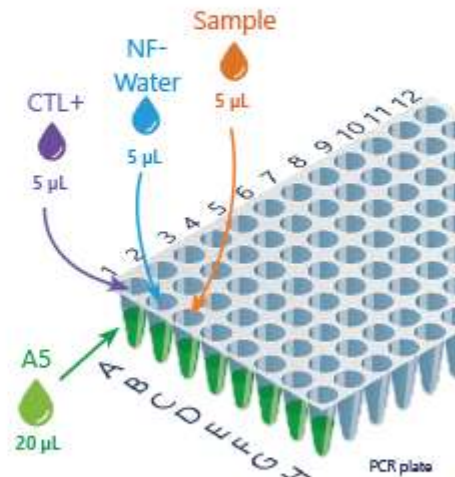
**Adia^X
Mag**



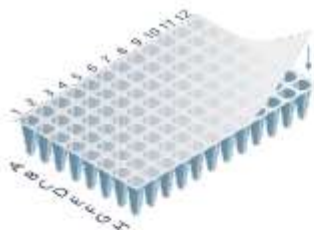
Scan me to
discover Adiamag™

2 | Distribute 20 μ L of A5 amplification
solution

3 | Distribute 5 μ L of nucleic acids, CTL+
and NF-Water

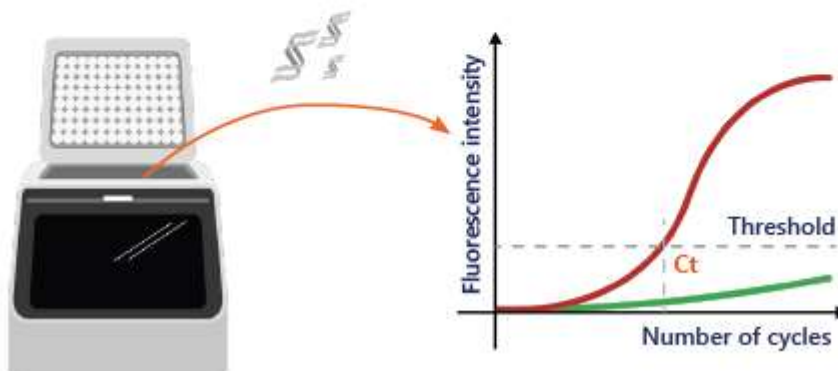


4 | Seal the wells



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5 | Start PCR analysis



*The notes do not replace the instructions for use of
which they are a summary.