Adia X Lyo

Instruction manual
ADL55Y2-ASFV_NO_(EN)_V01
01/2024

ASFV Triplex

Reference: ADL55Y2-100 & ADL55Y2-1000

Test for the detection of African Swine Fever Virus (ASFV) by real time enzymatic amplification PCR Test – 100 & 1000 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	✓	✗
Environmental sample	✓	✗

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

Kit composition

Content		ADL55Y2 Kit	
		100 reactions	1000 reactions
A6	Amplification solution	1 lyophilized vial with blank caps (To reconstitute)	10 lyophilized vials with blank caps (To reconstitute)
Rehydration buffer	Rehydration solution	1 x 6 mL vial (Ready to use)	2 x 6 mL vial (Ready to use)
ASFV CTL+	ASFV positive control	1 tube with purple cap (To reconstitute)	2 tubes with purple cap (To reconstitute)
EPC-Ext	Exogenous extraction or amplification control	1 lyophilized vial with yellow cap (To reconstitute)	6 lyophilized vials with yellow cap (To reconstitute)
NF-Water	Nuclease-Free Water	2 x 1000 µL tubes with white cap (Ready to use)	1 x 10 mL vial with white cap (Ready to use)

Revision history

Date	Version	Modifications
01/2024	V01	Creation

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 ASF 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

ADIALYO™ ASFV Triplex 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: internal control of extraction and amplification specific from an endogenous nucleic acid (HEX labelled probe or its equivalent).
- EPC-Ext: Internal control of extraction and/or amplification specific from an exogenous (Cy5 labelled probe or its equivalent).

C. Storage conditions

- Store the kit at a temperature below +2/8 °C after reception.
- Store away from sunlight and keep dry.
- After reconstitution, prepare aliquots and store them at a temperature below -15 °C until the expiration date.
- Do not thaw more than 3 times.

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 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.
- 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 or until 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.

Control	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. Amplification solution A6 preparation

- Add **1000 µL** of **« Rehydration buffer »** per A6 tube.
- Homogenize tube contents using a mixer, such as vortex, at least 20 seconds.
- After reconstitution, aliquot the solution and store at a temperature below -15 °C until the expiration date. Do not thaw more than 3 times.
- To use the A6, please refer to §« Amplification », Step 1.

2. Preparation of controls

a. Use of EPC-Ext

EPC-Ext must be added to each sample and controls.

- Add **1000 µL** of **« NF-Water »** per tube.
- Homogenize the tube contents using a shaker such as a vortex, > 20 seconds.
- 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 use, add **5 µL** of EPC-Ext in the first lysis buffer during the extraction of nucleic acids.

b. 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).

3. 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 **10 µL** of amplification solution (A6) 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: Set up the amplification program.

The following program is defined for ABI Prism thermocyclers (like 7500, QuantStudio5, 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.

DNA/RNA Program	
10 min. 45 °C	
2 min. 95 °C	
5 sec. 95 °C	40 cycles
30 sec. 60 °C*	

*Reading and parameters for fluorescence acquisition:

Fluorochrome	Absorbance (nm)	Emission (nm)
FAM	494	520
HEX or equivalent	538	554
Cy5	646	662
ROX	575	602

Note: The Quencher is non-fluorescent. The A6 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. Reading and interpretation

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 (ASFV)	Cy5 (EPC-Ext)	HEX (Endo-genous)	
No Template Control (NTC)	No	No	No	Absence of amplification contamination
ASFV CTL+	Yes	No	Yes	Target amplification
Extraction negative control	No	Yes	No	Absence of extraction contamination
Extraction positive control	Yes	Yes	Yes/No	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, Cy5 and/or HEX or equivalent.

Amplification			Interpretation
FAM (ASFV)	Cy5 (EPC-Ext)	HEX (Endogenous)	ASFV
Yes	Yes	Yes	Detected
Yes	No	Yes	Detected
Yes	Yes	No	Detected
Yes	No	No	Detected
No	Yes	Yes	Undetected
No	No	Yes	Not determined[1]
No	Yes	No	Undetected for acellular matrix
			Not determined[2]
No	No	No	Not determined[3]

« Undetermined »: No characteristic amplification curve for critical controls.
Possible causes:
[1] Extraction issue and/or PCR inhibition.
[2] Sample forgotten or degraded during extraction.
[3] Potential PCR error/inhibition or error during the extraction.
Recommendations:
Set up a new PCR assay using pure nucleic acids extracts and 10x dilutions in Nuclease-free water or perform a new nucleic acids extraction.

Symbols

Symbole	Signification
REF	Catalog number
	Manufacturer
	Temperature limitation
	Use by
LOT	Batch code
	Consult Instructions for Use
	Contain sufficient for "n" tests
VET	For veterinary *in vitro* use only – For animal use only
	Keep away from sunlight
	Keep dry

Notes

1 Extract nucleic acids with

Adia X Mag

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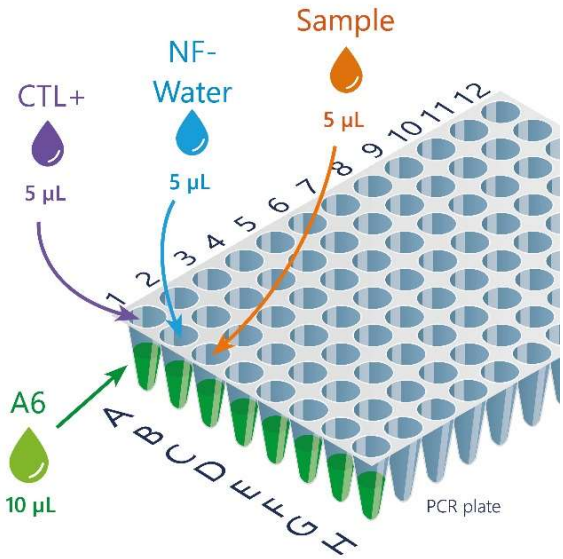
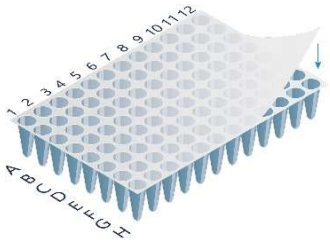
2 Add **1000 µL** of Rehydration buffer to the **A6** amplification solution

3 Distribute **10 µL** of **A6** amplification solution

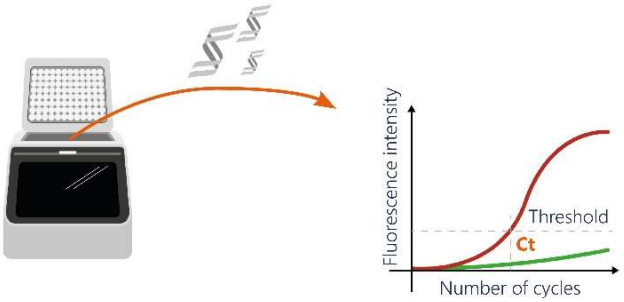
4 Distribute **5 µL** of nucleic acids, **CTL+** and **NF-Water**



5 Seal the wells



6 Start PCR analysis



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

Contact us
support.pcr@biox.com
www.biox.com +32 (0) 84 32 23 77
Bio-X Diagnostics
38, rue de la Calestienne
5580 Rochefort (Belgium)