EURx® MOLECULAR BIOLOGY PRODUCTS

Date of issue: 22.01.2021

For laboratory/veterinary use only,
for in vitro diagnostics

ASFV qPCR Detection Kit

Kit Components

Component	Cat. No. E0457-01 24 reactions of 25 µl	Cat. No. E0457-02 96 reactions of 25 µl
ASFV qPCR Master Mix (2x)	1 x 315 µl	4 x 315 µl
PC—positive control	1 x 25 µl	1 x 150 µl
Water, nuclease free	1 x 500 µl	4 x 500 µl

Storage

Store the components at -20°C in the dark.
Repeated thawing and freezing (> 2x) should be avoided, as this may reduce the sensitivity.

Description

- ASFV qPCR Detection Kit is a ready-to-use system for the detection of DNA from *African Swine Fever Virus* in serum, plasma, blood, tissue and swab samples from pig and wild boar.
- The kit contains reagents and primers for the amplification of a highly conserved ASFV p72 gene fragment. The detection of the ASFV amplicon is based on using the specific FAM-labelled probe.
- In addition, the kit contains a second heterologous amplification system to monitor DNA isolation and PCR reaction correctness. The inner control consists of primers and the HEX-labelled probe for porcine ACTB gene coding β-actin and present within the sample.
- ASFV qPCR Master Mix (2x) contains onTaq DNA Polymerase, optimized reaction buffer, dNTPs all necessary primers and probes required and ROX passive reference dye.
- onTaq DNA Polymerase is a modified "hot start" enzyme that is blocked at moderate temperatures and allows room temperature reaction setup.
- The polymerase activity is restored during the initial denaturation step when amplification reactions are heated at 95°C for 15 minutes.
- ROX passive reference dye included in the master mix allows fluorescence normalization on certain cyclers. The use of ROX dye is necessary for all real-time PCR cyclers from Applied Biosystems and optional for cyclers from Agilent. ROX compensates for variations of fluorescent signal between wells due to slight differences in reaction volume and fluorescence fluctuations. ROX is not involved in PCR reaction and does not interfere with real-time PCR on any instrument.
- The positive control includes the targeted viral DNA and porcine ACTB gene region and serves to prove functionality of the ASFV assay. C_T for the ASFV target in the FAM channel should be: 30±3, while for the ACTB target in the HEX channel should be: 29±3.
- Prior to real-time PCR, viral DNA must be extracted from the starting material with dedicated, commercially available kits using silica /magnetic beads technology.

Equipment and reagents to be supplied by user

1. Pipets
2. Sterile pipet tips with filters
3. Sterile 1.5 ml tubes
4. PCR tubes, strip tubes or 96-well optical microplates
5. Lab coat, disposable gloves, protective goggles
6. Centrifuge with rotor for 1.5 ml tubes
7. Centrifuge with rotor/adapter for 96-well microplates
8. Cooling device or ice
9. Real-time PCR cycler

Protocol

Preparation of PCR Reaction

Component	Negative Control 1 rxn	DNA Samples 1 rxn	Positive Control 1 rxn	Final concentration
ASFV qPCR Master Mix (2x)	12.5 µl	12.5 µl	12.5 µl	1 x
PC—positive control	-	-	5 µl	
Sample DNA	-	5 µl	-	
Water, nuclease free	12.5 µl	7.5 µl	7.5 µl	
Total volume	25 µl	25 µl	25 µl	

Notes:

1. A reaction volume of 25 µl should be used with most real-time cyclers. Other reaction volumes may be used if recommended for a specific instrument.
2. Thaw, gently vortex and briefly centrifuge all solutions. Keep the kit components on ice. Avoid multiple thawing and freezing (> 2x) of ASFV qPCR Master Mix (2x), as this may reduce the sensitivity.
3. Set up PCR reactions at room temperature. Use of ASFV qPCR Master Mix (2x) allows room temperature reaction setup.
4. Prepare a reaction master mix by adding ASFV qPCR Master Mix (2x) and water.
5. Mix the reaction mix thoroughly and dispense 20 µl into PCR tubes or plates.
6. Add 5 µl of water (negative control), 5 µl of the sample DNA or 5 µl PC-positive control to the individual PCR tubes or wells containing the reaction mix.
7. For every PCR run prepare at least 2 additional reactions: negative and positive controls. The negative control should be prepared first and the positive control should be prepared last.
8. Centrifuge briefly to settle down the reaction components and remove bubbles. Bubbles interfere with fluorescent detection.
9. Place the samples in the cycler and start the program.
10. Perform data analysis.

Thermal Cycling Conditions

Step	Temperature	Time	Number of Cycles
Initial Denaturation	95°C	15 min	1
Denaturation	95°C	15 s	40
Annealing/Extension	60°C	1 min	
Cooling	4°C	Indefinite	1

Notes:

1. Run PCR reactions using standard ramp rate (standard mode).
2. During the initial denaturation step onTaq DNA Polymerase is activated. onTaq DNA polymerase requires 15 min incubation at 95°C to restore activity.
3. Fluorescence detection should be set on the end of the extension step and executed using FAM and HEX channels. The FAM channel detects ASFV DNA and the HEX channel detects the ACTB gene region.

Results Interpretation:

Sample type	FAM channel	HEX channel	Result
Negative control	-	-	correct
Positive control	+	+	correct
Sample 1	-	+	Negative ($ASFV^-$)
Sample 2	+	+	Positive ($ASFV^+$)
Sample 3	+	-	Positive ($ASFV^+$)
Sample 4	-	-	incorrect

Notes:

1. For the assay to be valid the positive control must give a signal in both the FAM and HEX channels. The Negative Control must give no signal.
2. The sample is positive for ASFV and the assay is valid if:
   - the cryteria in the first point are met,
   - The sample yields a signal in both the FAM and the HEX channels.

   Exceptionally very high concentrations of ASFV DNA in the sample may lead to a reduced HEX signal or no HEX signal at all due to the competition with the internal control.
3. The sample is negative for ASFV and the assay is valid if:
   - the cryteria in the first point are met,
   - The sample yields a signal in only the HEX channel.
4. A positive HEX signal for the sample means that the DNA extraction and amplification were successful. High C_T values (>35) for internal control may indicate partial PCR reaction inhibition. In turn lack of a signal at all in the HEX and FAM channel simultaneously may indicate complete PCR reaction inhibition or that no DNA was added to the test sample. In this case it is recommended to dilute 10 times the sample DNA or repeat the DNA extraction and run the PCR reaction again.
5. Absence of a signal for the positive control indicates to an error, which could be due to incorrect setup of the PCR reaction.