Opti ASFV-qPCR (Instructions for use)

Real-time qPCR detection of African Swine Fever Virus

Catalog Number IA01

01. Product description

The OPTIPHARM Opti ASFV-qPCR (Cat. No. IA01) enables detection of the African Swine Fever Virus (ASFV) in swine blood, serum, or tissues by real-time qPCR amplification of the ASFV *P72* gene.

The qPCR Premix includes buffer, enzyme, primers and fluorescence probes for optimized real-time qPCR amplification of the ASFV and Internal control. Internal control is used to monitor for the presence of PCR inhibitors.

This product can be tested using CFX96 (Bio-Rad), ABI 7500, ABI QuantStudio5 (Thermo Fisher), Exicycler96 (Bioneer), Mx3005P (Stratagene Agilent), SmartCyclerII (Cepheid) real-time qPCR instruments (*Roche, not recommended).

02. Contents and storage

No.	Component	Amount	Storage[*]
1	qPCR Premix	8 T x 12 Strips	-20°C
2	Positive control	100 µL, 1 Vial	

[*] See packaging for expiration date.

03. Set up the PCR reactions

1. Thaw all reagent on ice and protect from light
2. Prepare DNA sample for testing, spin down the tube briefly.
3. Add 5 µL of template DNA to the 8-strip tube.
4. To the ASFV positive control reaction tubes, add 5 µL of the positive control.
5. To the negative control reaction tubes, add 5 µL PCR-grade H_2O (or negative extract).
6. Gently mix the reactions without creating bubbles (do not vortex) and then centrifuge briefly to bring the contents to the bottom of the plate wells or tubes.

04. Set up and run the real-time qPCR

1. Following the manufacturer's instructions, set up the real-time PCR run using the following parameters.
   1) Reaction volume: 20 µL
   2) Select detectors and assign Fluorescence probe reporter dyes and quenchers for each tube used in the analysis.

Target	Reporter	Quencher
ASFV	FAM dye	BHQ1
Internal control	HEX dye	BHQ1

NOTE: The FAM/HEX (VIC) should be detected by the same tube.

3) Thermal cycling program:

Step	Temperature	Time	Repetition
1	95°C (Pre-denaturation)	3 min	1
2	95°C (Denaturation)	3 sec	10
	60°C (Annealing)	30 sec	
3	95°C (Denaturation)	3 sec	40 (Plate read)
	55°C (Annealing)	30 sec	

05. Guidelines for data analysis

1. The Ct value of the result is significant within the range 35 or less.
2. The Ct value of positive control should be within the range 25 or less.
3. The Ct value of internal control should be within the range 30 or less.
4. Interpretation of results

Amplification signals	Interpretation
FAM positive HEX positive	ASFV is detected.
FAM positive HEX negative	ASFV is detected. (If the concentration of sample DNA is too high, internal control may not be detected.)
FAM negative HEX positive	ASFV is not detected.
FAM negative HEX negative	ASFV is not detected (If the concentration of sample DNA is too high or too low, Internal control may not be detected. *Retesting is required.)

06. Technical support

Use the information below to inquire about product specifications or technical issues.

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