

Genelix™ ASFV Real-time PCR detection kit

Cat. No. G701/G702



Validated and certified by the WOAHA as fit for the purposes defined in the kit insert provided with this kit. Registration number 052131

Please read instructions carefully before you perform the test

Intended Use

The Genelix™ ASFV Real-time PCR Detection kit is a product that qualitatively detects and confirms the diagnosis of ASFV using a real-time PCR detection system in the whole blood, serum, and tissues of swine suspected of being infected with the ASFV.

Principles

Genelix™ ASFV Real-time PCR Detection kit is based on TaqMan probe real-time fluorescent PCR technology. Target DNA is used as a template for PCR amplification. During the PCR reaction, the polymerase activity and exonuclease activity of Taq DNA polymerase were simultaneously used. During exonuclease activity, the fluorophore and quencher attached to the TaqMan probe were cleaved. PCR instrument detects the fluorescence generated by degradation of the probe.

Kit Components

1. Genelix™ ASFV Real-time PCR Detection kit (G701, Strip type, 48 rxn)

Contents	Quantity
Real-time PCR Premix	Each tube 15 µL
Positive Control DNA	30 µL/tube
PCR Flat Cap	6 ea
Insert	1 ea

2. Genelix™ ASFV Real-time PCR detection kit (G702, General type, 100rxn)

Contents	Quantity
Real-time PCR Master mix	1,100 µL/tube
Primers/Probe Mixture	550 µL/tube
Positive Control	60 µL/tube
Negative Control	60 µL/tube
Insert	1 ea

Instruments for the kit

Instruments	Manufacture
Bio-rad CFX96™	Bio-rad
AB 7500 Real-Time PCR System	ThermoFisher
AB 7500 Fast Real-Time PCR System	ThermoFisher
QuantStudio™5 Real-Time PCR System	ThermoFisher

Kit Storage and Stability

All reagents of the Genelix™ ASFV Real-time PCR Detection kit must be stored at -20 ± 5 °C. Kit components are stable until the expiration date printed on the outer packaging. The components of the Genelix™ ASFV Real-time PCR Detection kit should be stored away from light. Freezing-thawing of kit components more than six times may lead to inaccurate results.

Warning and Precautions

A. General Warnings

- For professional use only.
- For veterinary *in vitro* diagnostic use only.

- This test kit is intended for diagnostic use and should be used by experts such as pathologists or veterinarians.
- Follow the guidelines and procedures described in this 'Instructions for use' to achieve optimal results.

B. Safety Precautions

- Please operate according to the laboratory testing procedures for infectious diseases.
- All specimens must be considered potentially infectious and handled with appropriate biosafety practices.
- Do not eat, drink, or smoke in the laboratory.
- Personal protective equipment (gloves, goggles, and lab-gown) should be applied during specimen processing and reagent preparation.
- Wash hands thoroughly when finished.
- Thoroughly clean and disinfect all work surfaces with 1 % sodium hypochlorite.
- Waste, used kit materials, and tools should be treated as biohazardous waste in accordance with regional disposal regulations.

C. Analytical Precautions

- Do not use the test kit beyond the expiry date mentioned on the package label.
- Do not use the kit if the solutions leak, are damaged, or are broken.
- Do not use specimens stored for too long or contaminated.
- The provided material is optimized for the kits in the same box. Do not use reagents from other sources or different lots.
- The strip-tube in G701 is disposable. Do not reuse.
- All consumables should be thoroughly sterilized; reuse is prohibited.
- Thaw and mix reagents properly before reagent preparation. Repeated freezing and thawing of reagents/specimens should be avoided because this may affect test performance.
- Beware of microbial contamination when dividing solutions. The use of a sterilized disposable filter tip is recommended.
- Periodically calibrate the measuring instruments. (e.g., pipettes, etc.) Accurately instill the solution for a precise result.
- When mixing the reacting solution in the PCR tubes, vortex and then spin down before use. Carefully avoid bubbles in each strip.

Specimen Collection and Preparation

- Specimen types: Swine whole blood, serum, and tissues with suspected ASFV infection.
※ **NOTE: All samples must be treated as potentially infectious substances.**
- It is recommended that specimens shall be used immediately after collection. If immediate use is not achievable, the specimens can be stored for a few days at 4 °C in a fridge or more than seven days at colder than -70 °C in a deep freezer.
※ **NOTE: Do not repeat freezing and thawing.**

Nucleic acid Extraction

Sample preparation and nucleic acid extraction should be carried out in a shielded laboratory. The use of a commercial extraction kit following the user manual provided by the manufacturer is recommended.

Commercial Extraction kit	Manufacture
Genelix™ Viral DNA/RNA Extraction kit	Sanigen co., Ltd
High Pure PCR Template Preparation Kit	Roche Molecular Systems, Inc.
AccuPrep Genomic DNA Extraction kit	Bioneer
DNeasy Blood & Tissue Kits	Qiagen

■ Reagent preparation

Prepare PCR mix solution(s) considering the number of samples to be tested. Thaw the strip tube in ice or use a laptop cooler to prevent heat damage. Thaw the strip tube in ice or use a laptop cooler to prevent heat damage.

To prevent deformation, divide the positive control into several tubes when first opened and store them in the freezer.

[G701]

- i. Remove the dome cap and add 5 µL of extracted samples into the PCR Premix strip tube.
- ii. Prepare a positive and a negative control with the same procedure as the sample.
- iii. Seal the tube using the flat cap (provided with the kit) and spin down the mixed reagent.
- iv. Prepare 3 types of samples as indicated below. The quantity of ① depends on the number of samples to be tested.

Composition example	Volume/run
① PCR Premix 15 µL + Extracted sample 5 µL	20 µL
② PCR Premix 15 µL + Positive control 5 µL	20 µL
③ PCR Premix 15 µL + Negative control 5 µL	20 µL

[G702]

- i. Take 10 µL of PCR Master mix solution in a PCR tube and add 5 µL Primer/Probe Mixture.
- ii. To prepare the sample reaction mix, add 5 µL of extracted samples and mix the reagent.
- iii. Similarly, prepare positive and negative control reaction mixtures.
- iv. Seal the tubes and spin down the mixed reagent.
- v. The composition of the 3 types of reaction mixtures are listed below. The quantity of ① depends on the number of samples to be tested.

Composition example	Volume/run
① Master mix 10 µL + Primer/Probe Mixture 5 µL + Extracted sample 5 µL	20 µL
② Master mix 10 µL + Primer/Probe Mixture 5 µL + Positive control 5 µL	20 µL
③ Master mix 10 µL + Primer/Probe Mixture 5 µL + Negative control 5 µL	20 µL

■ PCR Amplification

Place the tubes in a real-time PCR machine and start the machine, setting the following conditions. The fluorescence curves are analyzed in the FAM channels.

Temperature	Time	Cycle
50°C	2min	1
95°C	10min	1
95°C	15sec	40
60°C	1min	

■ Interpretation of Results

The criteria for setting threshold and baseline according to the equipment are as follows.

Instrument	Threshold	Baseline start	Baseline end
AB 7500	0.1	3	15
AB 7500 Fast	0.1	3	15
QuantStudio™ 5	0.4	3	15
Bio-rad CFX96™	100	3	15

If the positive and negative control results match the following criteria listed in the table, interpret the results for the target sample(s). If the results of the control materials do not match the table, set the experiment again.

Control type	Ct value
Positive control	Ct ≤ 38.1
Negative control	Non-Detected

Check the Ct value of the sample(s) using the instrument-specific software. The sample data is considered positive at Ct ≤ 38.1 and negative at Ct > 38.1.





Positive control	Negative control	Sample	Interpretation
-	-	+/-	Invalid
+	+	+/-	Invalid
-	+	+/-	Invalid
+	-	+	ASFV positive
+	-	-	ASFV negative

※ NOTE: As with all diagnostic tests, all results must be considered with other clinical information available to the veterinarian.

■ Troubleshooting

Trouble	Cause	Suggestions
Positive signal at Negative Control	Carry-over contamination	Take care to avoid contamination.
No or weak signal at Positive Control	Insufficient quantity of the positive control reaction mix.	Check the amount in the positive control tube.
	Positive control reaction mix has been lost or degraded.	Positive control should be divided into aliquots of the appropriate volume required for a single test and stored properly to avoid repeated thawing.
Abnormal results in fluorescence signal intensity	Dispensing amount error	A certain amount of sample(s) should be tested.
	Contamination in test equipment or environment	Be cautious to avoid contaminating the sample(s).
	Unstable dispensing state of solution (such as bubbles)	Spin down the solution to remove the bubbles.
Weak signal in positive control and all sample	Improper storage conditions	Store under appropriate conditions.
	PCR software error	Check the reaction conditions.

■ Symbol Information

Symbol	Meaning
	Catalog number
	Contains sufficient for <n> tests
	Temperature limit
	Manufacturer



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