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African Swine Fever Virus (ASFV) Nucleic Acid Detection Kit

(Fluorescence PCR Method)

User Guide

FOR ANIMAL USE ONLY!

Σ P708H

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Version 1.0

Qualitative In-Vitro Diagnostics/For Use with qPCR Instruments Compatible with
African Swine Fever Virus (ASFV) Nucleic Acid Detection Kit (Fluorescence PCR Method)

REF P708H

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Introduction

African swine fever (ASF) is an acute, hemorrhagic, and high contact infectious disease of pigs caused by African swine fever virus (ASFV). Swine usually show clinical symptoms such as high fever, respiratory failure, diarrhea after ASFV infection, and punctuate hemorrhages can be observed in the skin and internal organs after necropsy of some pigs, whereas pregnant sows often experience abortion and stillbirth, etc. ASFV is highly resistant to the outside world and can persist in blood, secretions, and various environmental pollutants for a long time. ASFV has a large genome and intricate immune escape mechanisms that can be well evaded by the host immune cell clearance, so there are no safe and efficient vaccines and therapeutics, and the way to control the spread of this virus is simply to cull infected animals. Therefore, rapid and accurate diagnosis of ASF is very important, the ***African Swine Fever Virus (ASFV) Nucleic Acid Detection Kit (Fluorescence PCR Method)*** developed by Xi'an Tianlong Science and Technology Co., Ltd. assist in the diagnosis of ASFV and public healthcare management.

Intended Use

The Tianlong's ***African Swine Fever Virus (ASFV) Nucleic Acid Detection Kit (Fluorescence PCR Method)*** is intended to be used for the qualitative detection of ASFV nucleic acid by fluorescence Polymerase Chain Reaction (PCR) method.

The test is designed to detect DNA from ASFV in specimens such as cell free body fluid samples, whole blood, serum, tissue or environmental samples.

The Tianlong's ***African Swine Fever Virus (ASFV) Nucleic Acid Detection Kit (Fluorescence PCR Method)*** is to be used with Real-time PCR instruments with 2 or more fluorescence detection channels, which the test performance of the kit has been validated on such Real-time PCR thermal cyclers have appropriate fluorescence reading channels for FAM, VIC, etc. The reagent has been validated on Tianlong Gentier Real-time PCR system, Roche cobas z480, Applied Biosystems™ QuantStudio™ 5 Real-Time PCR System and Applied Biosystems™ 7500 Real-Time PCR Systems.

Note: ABI QuantStudio 5 and ABI 7500 need to add additional ROX reference dye to the system, the dye is s elf-prepared, and the final concentration of 30-50 nM is recommended.

The test results are for clinical reference only and cannot be used as the basis for confirming or excluding cases alone.

Content of the Kit

Short Code:		**P708H**
Number of reactions		50 Tests
RT-PCR reagents		
ASFV Fluorescence Reaction Buffer	750 µL	1 tube
ASFV Primer and Probe Mixture	250 µL	1 tube
Controls		
ASFV Positive Control	50 µL	1 tube
ASFV Negative Control	50 µL	1 tube
Instructions for Use		1 Copy

Note: Store all reagents between -25°C to -15°C in a non-frost-free freezer. Do not mix the reagents from different batches. The negative control can be referred as a "No Target Control" (NTC).

Materials Required but not Provided

- Microliter pipets* dedicated for PCR (0.1-2.5 μL; 1-10 μL or 1-20 μL; 20-200 μL;1000 μL).
- Benchtop centrifuge* with rotor for 0.5 mL/1.5 mL reaction tubes.
- Benchtop vortex mixer*.
- PCR instrument* with FAM, VIC channels, etc.

(*): Ensure that instruments have been checked and calibrated according to the manufacturer's recommendations.

(*): Please refer to relevant manufacturer's Instructions for Use (IFU) to proceed with the instrument.

Principles of the Assay

The kit is designed with specific primer and specific probe on African Swine Fever Virus (ASFV) conserved gene. The probe will have specific binding with one section of DNA template in the middle of primer amplification area. In PCR extension reaction process the excision enzyme activity of Taq enzyme will cut down 5'-end fluorophore from probe to make it free in reaction system and break away from shielding of the 3-end fluorescence quencher, which means it can accept the optical excitation, emit fluorescence for instrument test and achieve automatic test for ASFV nucleic acid in totally enclosed reaction system by this wave. One pair of housekeeping gene specific primer was selected and used in the internal control of the kit, combined with the specific probe which can combine with one section of DNA template in the middle of primer amplification area. VIC channel was selected for internal control to achieve monitoring for test process in a totally enclosed reaction system and effectively monitoring for occurrence of false negatives.

Sample Requirements

1. Sample Source: As stated in the intended use**.**

2. Sample treatment: Separate treatment of each sample.

(1) Cell free body fluid sample treatment: Collect at least 400 μL sample and transfer it into a 1.5 mL centrifuge tube.

(2) Whole blood sample and serum sample treatment: Take at least 400 μL whole blood sample or serum sample and transfer it into a 1.5 mL centrifuge tube.

(3) Tissue sample treatment: Weigh about 1 g of each tissue sample from three different locations and cut it into pieces with surgical scissors and mix well. Then, put 0.05 g into the grinder, and 1.5 mL normal saline and continue to grind it thoroughly. After homogenization, transfer it to a 1.5 mL sterilizing centrifuge tube, centrifuge it at 12,000 rpm for 5 min, take 400 μL supernate and put it into a 1.5 mL centrifuge tube.

(4) Environmental sample treatment: Centrifuge it at low speed for 1min, take 400 μL supernate and put it into a 1.5 mL centrifuge tube.

3. Storage: Samples can be stored at 2~8°C for no more than 24 hours.

4. Transportation: Use a foam box with ice to seal for transportation.

Reagent Storage and Handling

All reagents must be stored at-25°C to -15°C for 12 months.

The stability of unspent reagents would not be influenced by re-storage. But the thawing and freezing should not be more than three times.

The opened reagents should be placed no more than 8 hours at room temperature. The products should be shipped by ice box or refrigerated truck under 2°C to 8°C. Simulated transport tests indicate that stability and validity could not be influenced by transport.

Starting

- Identify the product.
- Verify the expiration date.
- Verify the latest instructions for use available for the product lot number.
- Verify if the product was used already. If yes, check the remaining tests available.

RT-PCR Reaction Setup

1. Thaw the following reagents on ice: ***ASFV Fluorescence Reaction Buffer*** and ***ASFV Primer and Probe mixture***. Gently and evenly mix each individual reagent, then briefly centrifuge (2000 rpm, 10 s) the reagents to collect the contents.
2. Set up a premix solution based on the number of sample preps to be tested. The volume of the premix required for all sample prep(s) to be tested= (number of sample preps + 2 controls) *the total volume of premix reagents (listed in Table 1).

Table1: Premix Reagents

Premix Reagents	Volume
ASFV Fluorescence Reaction Buffer	15 µL
ASFV Primer and Probe mixture	5 µL
Total volume	20 µL

3. Evenly aliquot 20 µL of the premix Reagents into each PCR tube (one PCR tube per sample to be tested). Add 5 µL of each extracted DNA solution to a single PCR tube. Do not add more than one sample of extracted DNA into a single PCR tube. Add 5 µL of ASFV Positive Control and Negative Control to the respective distinct PCR tubes (Positive Control and Negative Control do not require extraction). Each PCR tube shall have a total volume of 25 µL. Then immediately close the tubes and transfer the reaction setup into a PCR machine for the amplification.

Thermal Cycler Settings

Set up the following thermal cycling program. It is recommended to use a 2-channels PCR system.

Table2: PCR Cycling Program

Stage	Cycle	Temperature (°C)	Time (min: s)
1	1	37	2:00
2	1	95	3:00
3	45	95	00:15
		60	00:30 (Fluorescence collection)
4	1	25	00:10

Selection of fluorescence channels: ASFV (FAM) and internal controls (VIC).

Detection Channels

Two channels are used in this one-tube PCR assay. It is recommended to perform the colour (channel) calibration as requested by the instrument's manufacturer. Please refer to the instrument's user manual to perform this calibration.
Threshold Value Setting principle:

- Manual setting: Set the threshold value a little bit greater than the max fluorescence value of the normal negative control amplification curve.
- Auto setting: The instrument automatically sets the threshold value.

Result Interpretation

After the above quality control conditions are met, carry out the following analysis (ASFV in the FAM channel and internal control in the VIC channel):

FAM (ASFV)	VIC (Internal Control)	Result
Ct<45	Ct ≤35	ASFV Positive
No Ct value	Ct ≤35	ASFV Negative
No Ct value	No Ct value or Ct>35	**It is an invalid test** and needs to be checked and retested.

Performance Characteristics

The following performance characteristics of the Tianlong's ***African Swine Fever Virus (ASFV) Nucleic Acid Detection Kit (Fluorescence PCR Method)*** have been established following the procedure described in this datasheet.

Non-clinical Studies

- Limit of detection:500 copies/mL.
- Specificity: There was no cross-reaction of other common pathogens with the same infection site or similar infection symptoms.
- Precision: The assay was used to respectively detect the precise reference specimens of high and low concentrations in different time ranges 20 times, and the precision values of intra and inter Ct values were all <5%.

Limitations

Limits

- All reagents in the kit are intended for in vitro diagnostic use as indicated.
- The test should be carried out by professionals adequately trained in professional lab practices. It is the user's responsibility to verify/validate the testing system performance in their respective laboratory settings. Expired reagents should not be used.
- Strict compliance with the IFU is required for optimal results. Deviation from standard procedures during sample collection, preservation, transportation, processing, and testing could lead to false negative or false positive testing results.
- The test results are related to the conditions of specimen collection, storage and transportation. False negative results can be caused by any mistake in any link. False positive results may occur if cross-contamination occurs during specimen treatment.
- The test results cannot be directly used as the basis of clinical diagnosis or exclusion of cases, only for the reference of clinicians.

Warnings and Precautions

Laboratory Precautions

Use extreme caution to prevent:

⚠ **DNase contamination which might cause degradation of the template DNA; DNA or PCR carryover contamination resulting in false positive signal.**

We therefore recommend the following:

- To make sure an accurate and reliable result, always use DNase/RNase-free disposable pipette tips, tubes and calibration pipettes.
- Use separated and segregated working areas: 1) Reagent preparation area -preparing the reagents for amplification, 2) sample preparation area-isolation of the RNA/DNA from sample and control, and 3) Amplification area-amplification and detection of nucleic acid target.
- To avoid contamination, all the objects should be used in certain areas. All apparatus must be cleaned after each experiment.
- To avoid the contamination of fluorescent materials disposable gloves, tubes, pipettes and filter tips should not contain fluorescent material.
- Avoid the bubbles when separating the reaction solution into tubes. Check the tubes before amplification to avoid contamination induced by the leak of fluorescent materials.
- Nucleic acid samples stored at -20°C should be thawed mixed and centrifuged at low temperature for a short time before use.
- The reaction tube containing the reaction solution should be capped or packed in a sealed bag and then transferred to the sample processing area.
- When adding the sample, the sample should be completely added to the reaction solution and no sample should adhere to the tube wall. The tube cap should be closed as soon as possible after the sample is added.
- Try to avoid the generation of air bubbles when the reaction solution is dispensed, and check whether the reaction tubes are tightly closed before loading on the machine to avoid leakage contaminating the instrument.
- After the amplification the reaction tube was taken out, sealed in a special plastic bag, and discarded at the designated place.
- The used tips should be thrown into disposal bottle which have 10% sodium hypochlorite solution and discarded with other waste.
- Use 10% sodium hypochlorite, 75% alcohol and ultraviolet light to disinfect the workbench and experimental items regularly.
- The real-time PCR instrument requires frequent calibration and cleaning of the wells of the plate.

Safety Symbols and Signs

No.	Symbol	Implication
1	REF	Catalogue number
2	LOT	Batch code
3	Σ <N>	Contains sufficient for <N> tests
4		Use by date
5	⚠	Caution

6		Temperature limit
7		Manufacturer
8		Date of manufacture
9		PAP21: Non-corrugated fibreboard

Contact Information

For technical assistance and more information, please contact our Technical Support Center at +86-29-82682132 (Tel), +86-29-82216680 (Fax), inquiry@medtl.com or contact your local distributor.
For up-to-date licensing information or product-specific disclaimers, please see the respective User Guide. Tianlong User Guides are available at www.medtl.net or can be requested from Tianlong Technical Services or the local distributor.