(Fluorescent Probe Method-PCR) Nucleic Acid Detection Kit (ASFV) for African Swine Fever Virus

[Product Name]

Common Name: African Swine Fever Virus ASFV Kit (Fluorescent Probe Method)

[Packaging Specifications]

50 Detection Servings/Box

[Intended Use]

This kit is used to qualitatively detect African swine fever virus (ASFV) in P72 gene

African swine fever is an acute, severe, and highly contagious infectious disease of pigs caused by African swine fever virus. The morbidity and mortality of African swine fever can reach100%. It has seriously endangered the global pig industry and caused incalculable economic losses. The World Organization for Animal Health (OIE) lists it as a statutory reportable animal disease, and China lists it as a Class I animal disease.

[Test principle]

This kit adopts fluorescent PCR method for nucleic acid detection. ASFV genome P72 is used as the detection target region, and specific primers and Taqman probes are designed, together with PCR reaction solution and other components on the instrument, the real-time fluorescence quantitative PCR African swine fever virus DNA the rapid detection of This kit uses porcine ATCB gene as the internal quality control, and monitors the collection, transportation and extraction process of the samples to be tested through the internal standard to avoid false negative test results.

In addition, PCR detection system UNG enzyme + dUTP can effectively degrade the aerosol pollution of amplification products and avoid false positives.

【Main components】

Serial number	name	specification and quantity
1	ASFV amplification reaction solution	× 1tube
2	Positive control	100 µL × 1tube
3	Negative control	100 µL × 1tube

1000µL! This kit does not contain the necessary extraction reagents for detection.

[Storage conditions and validity period]

-20±5℃; the validity period of the kit is12months, please use it within the validity period, and the performance of the kit will not be affected after opening.

[Applicable instrument]

Nucleic acid amplification instrument: Anitoa series fluorescence quantitative PCR with the same performance PCR.

【Sample requirements】

1 Applicable sample types: pig tissue, serum, plasma, oral and nasal swabs and other samples.

2 Specimen collection

2.1 Tissue: Use sterile scissors and tweezers to collect the organs to be inspected and put them into sterile sampling bags or other sterilized containers.

2.2Serum: Use a disposable sterile syringe to draw5ml, wait for the sample to separate out serum, or directly2000rpmcentrifuge5minutes, suck the supernatant (be careful not to inhale red blood cells) and transfer it to a sterile centrifuge tube for use.

2.3 Plasma: Use a disposable sterile syringe to draw5mlof porcine venous blood into a blood collection tube containing EDTA anticoagulant (heparin should not be used), then gently invert and mix until the sample separates out of plasma, or directly 2000rpm centrifuge 5 minutes, transfer the supernatant to a sterile centrifuge tube for later use.

2.4 swab: Use a dry cotton swab with a length of15-20cm, go deep into the bottom of the nasal cavity, and rotate2-3times. After sampling, add1mLof normal saline to the test tube.

3 Specimen storage and transportation: The samples to be tested should not be stored at2-8°Cfor more than three days;-20±5°Cfor no longer than1month;at -70°Cfor long-term storage, repeated freezing and thawing should be avoided.

[Test method]

1. Reagent preparation (reagent preparation area)

Take out each component in the kit from the kit, place it at room temperature, wait for the temperature to equilibrate to room temperature, shake and mix well, centrifuge20uL/, and dispense PCR reaction tube for use.

2 Sample processing (sample processing area)

2.1 Nucleic acid extraction

Refer to the product instructions of the manufacturer of the nucleic acid extraction or purification kit for operation.

2.2 Add samples

prepared above PCR each of the processed sample nucleic acid, blank control, and positive control to the5 μ lfinal volume is25 μl/tube, close the tube cap tightly, and centrifuge at low speed for a while. In order to prevent the fluorescent signal from being disturbed, try to avoid touching PCR reaction tube with your hands.

3 PCR amplification detection (nucleic acid amplification area)

3.1 Put the reaction tube into the fluorescence quantitative PCR amplification instrument for amplification detection.

3.2 Put the PCR reaction tube into the sample tank of the instrument, set the experiment name, hole position information, select channel1(FAM) and channel4(CY5) for the detection channel, and then set the reaction conditions according to the following table:

step	temperature	time	cycle number
UDG enzyme Reaction	37°C	60s	1
Pre-denaturation	94°C	60s	1
Denaturation	94°C	5seconds	40
Annealing, extension	58°C	5seconds (acquisition of fluorescence)	
Instrument cooling	40°C	10seconds	1
Fluorescence detectionFAMandCY5			

Note: Due to ABI Step One instrument, annealing , The extension time cannot be set to5s, but can be adjusted to10s.

3.3 Result analysis

After the reaction is over, the instrument automatically analyzes the amplification curve and saves the results.

[Interpretation of results]

1 quality control standard

kit provides internal standard, positive control, and negative control. The same experiment must meet the requirements of the following table at the same time, otherwise, the experiment is invalid and needs to be repeated.

	Detection channel	normal result (Ct value)
negative control	FAM	no typical sigmoid curve, no Ct value
	CY5(internal standard)	

positive control	FAM	has typical sigmoid curve, Ct value<32
	CY5(internal standard)	

2 The result is judged

when the instrument is normal, the positive control and the negative control are normal The following results were analyzed:FAMchannel was the African swine fever virusP72gene, and theCY5channel was the internal quality control.

detection channel	gene	results for
FAM	p72gene	1.Positive: Ct value of the sample test result is less than or equal40, and the curve is S-shaped, which is judged as positive; 2.Negative: the sample test result has no Ct value or is negative, and it is judged as negative;
CY5	internal standard gene	The internal standardCY5channel detection result of the sample should be Ct≤40, otherwise the sample will be re-tested. When the sample result is interpreted as positive, if the internal standard Ct>40 or not detected, the result is still credible.

[Limitations of testing methods] The

results of sample testing are related to the quality of sample collection, processing, transportation and storage, and any mistakes in them will result in inaccurate results. False-positive results may occur if sample handling is not controlled for cross-contamination.

【Notes】

1. This kit is for research use only, please read this manual carefully before use.

2. Before the experiment, please be familiar with and master the operation methods and precautions of the instrument to be used and conduct quality control for each experiment.

3. Experimenters must undergo professional training, and all consumables used should be disposable.

4. Laboratories using this kit should manage in strict accordance with relevant national regulations.

5. the order of negative control, nucleic acid of the sample to be tested, and positive control PCR reaction tube in

6. Avoid generating air bubbles as much as possible during sample addition. After adding samples, briefly centrifuge to remove air bubbles. Check PCR reaction tube is tightly closed before loading the machine to avoid inaccurate results caused by liquid evaporation.

7. The instruments and wastes used for sample processing should be autoclaved. Consumables such as centrifuge tubes and suction tips used in the testing process should be directly injected into the waste liquid tank filled with disinfectant.

8. Immediately remove the PCR reaction tube after amplification, and seal it in a zip lock bag for harmless treatment.

9. During the experiment, it is strictly forbidden to use the gloves contaminated by the outer wall of the container and box to carry out the experiment.

10. Items in each area are dedicated and should not be used interchangeably to avoid contamination. The workbench should be disinfected immediately after the experiment.

11. The workbench and laboratory supplies should be cleaned and disinfected regularly.

[Manufacturing enterprise]

Name of manufacturing enterprise: Anitoa Biotechnology (Hangzhou) Co., Ltd.

Bin'an Road, Changhe Street, Binjiang District, Hangzhou City, Zhejiang Province658, No.2 Building 2nd Floor 210 Room

Tel:0571-85155309

[**Manual Approval Date and Modification Date**】2021__July 23,