African Swine Fever Virus (ASFV) Real-time PCR Kit

ASFV qPCR

Product Number: 0964-ZP01340-50T

Product Unit: 50T

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For veterinary use only.

1. Introduction

The African Swine Fever (ASF) is an acute, febrile infectious disease of pigs caused by ASFV, the fatality rate is up to 100%. This kit uses fluorescence probe PCR (Real-time PCR) method to detect ASFV, realized the qualitatively or semi-quantitatively detection of ASFV nucleic acid through real-time monitoring of fluorescence signal changes in the PCR process, this method is rapid and accurate, which is helpful for diagnosis, monitoring and epidemiological investigation of ASF.

2. Kit components

Item No	Description	Quantity
1	PCR reaction mix	1 tube (1 ml/tube)
2	Lysis solution	5 tube (1ml/tube)
3	Negative control	1 tube (10 μl/tube)
4	Positive control	1 tube (10 μl/tube)
5	Manual	1 piece

3. Application

This Real-time PCR kit can be used to detect ASFV nucleic acid in porcine blood, muscle, liver, lymph nodes, spleen, and feed samples.

4. Sample treatment and nucleic acid extraction

4.1 Sample treatment

Tissue: collect 0.1g samples from bovine tissue, lymph nodes, spleen, cut into pieces, then grind with 1.5ml of normal saline, then transfer it into 1.5ml sterile centrifuge tube, centrifuge for 2min at 12000rpm, store the supernate,

Blood: do not need any treatment, take 200ul of blood for later extraction.

Non-porcine sample: take feed or environmental sample into tube, add 1ml of PBS, ground it, then centrifuge for 2min at 12000rpm, store the supernate.

4.2 Extraction

1) Prepare 1.5ml of sterile centrifuge tubes according to the numbers of samples,
2) Add 90ul of lysis solution per tube.
3) Then add 10ul of treated sample into the tube, mix thoroughly, store at room temperature for 2min.
4) Centrifuge it for 1min at 12000rpm, the supernate the nucleic acid sample.

Note: Commercial test kit is recommended in the extraction of nucleic acid.

5. PCR amplification

- **Reagents preparation**

Remove the reagents from the refrigerator and restored them to room temperature for 20 minutes before the experiment, then it will melt completely. Then dilute the PCR reaction mix with 1ml of nuclease free water, and then centrifuge it to remove the liquid adsorbed on the tube wall. In addition, the negative or positive control should be diluted for 10 times (10ul positive/negative control + 90ul of nuclease free water) before using.

- **Reaction system**

The reaction system is established according to the table below, cover the tube tightly, then centrifuge for 10 seconds.

Components	**Volume**
PCR reaction mix	20 μl
Pre-extractive nucleic acid sample (sample/control)	5 μl
Total volume	25 μl

- **PCR amplification**

After preheating the machine and testing the performance of the instrument, place the PCR reaction tube (containing the above reaction system) into the sample cell, record the order, set the amplification parameters according to following table, and conduct PCR amplification.

Stage	**Temperature**	**Time**	**Repetitions**
1	95 ^{0}C	3 min	1
2	95 ^{0}C	5 s	40
	60 ^{0}C	15 s	
FAM channel			

6. Result Determination

Adjust the threshold value line, something will be different based on various instrument.

Validation Criteria

Reaction type	**ASFV target**	**Interpretation**
Positive control	Ct <= 35	PCR is validated
Negative control	No Ct	PCR reagents are validated

Interpretation of results

ASFV target	Result	Interpretation
Ct<=38	Positive	ASFV is detected
38<Ct<=40	Doubt	Second test is needed, if 38<Ct<=40, the result is positive, else negative
No Ct	Negative	ASFV is not detected

7. Precautions

- Read the kit manual carefully before use, you need perform the array following the steps strictly, otherwise could lead to wrong result.
- All the reagents should be stored under the requested temperature, reagents stored at -20 ^{0}C should be mixed fully before using.
- The test should follow the national laboratory administration standard, the experiment situation should be separated (reagents preparation section, sample preparation section, nucleic acid amplification section), avoid contamination.
- Operation bench, pipette, centrifuge and other instruments should be treated with 1% sodium hypochlorite, 75% alcohol or UV lamp. Other material should overnight treat with 0.1% DEPC.
- The samples, kit components and the waste produced in the experiment should treat in the way of infectious substrate.
- Do not use the gloves contained fluorescence, don't touch the experiment materials if you don't have a gloves.
- You need confirm it by other methods if your result is negative.
- The possible reasons of false negative: improper operation of the samples during collection, transportation, preservation and nucleic acid extraction, which could cause the DNA degradation; The nucleic acid concentration in the samples was lower than the minimum detection limit; Mutation of virus target gene; Other interference factors not verified.
- The possible reasons of false positives: cross contamination during sample collection, transportation and nucleic acid extraction.

8. Storage and expiration

The kit shall be store at -20 °C, avoid direct sunlight.
The valid period is 12 months.

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