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ASFV DNA qPCR Detection (High Sensitivity) Kit

Composition

ASFV DNA qPCR Detection (High Sensitivity) Kit	50 Preps
Cat. No.	7808050
Nucleic Acid Purification Kit	50 Preps
Nucleic Acid Amplification Reagent	
ASFV (African Swine Fever Virus) PCR Main Reaction Solution	1000 µl×2
Taq DNA polymerase Mixture	60 µl×1
Control Product	
Negative Control	50 µl×1
Positive Control (Containing Plasmid DNA That Detects Fragments Of DNA)	50 µl×1

Storage

The reagent can be stored at -20℃ for 1 year. The reagents should not be repeatedly frozen and thawed, should be completely thawed at room temperature before use, and fully mixed by inverting a few times.

Technical Support

R&D Department, Hangzhou Simgen Biotechnology Co., Ltd. E-mail: technical@simgen.cn, Tel: 400-0099-857.

Introduction

This kit uses a pair of specific primers of African swine fever virus (ASFV) DNA, a specific fluorescent probe, and uses Taq DNA polymerase, four monomer nucleotides (dNTPs) and other components, and uses PCR technology to achieve the amplification conservative gene of ASFV DNA. To detect ASFV DNA from pig serum or tissue homogenate supernatant.

Equipment and Reagents to Be Supplied by User

1. 1.5 ml centrifuge tubes, 8-tube strips or single tube PCR tubes.
2. Pipettes and tips (To avoid contamination between samples, please choose pipette tips with filter).
3. Disposable gloves and protective equipment and paper towels.
4. Microcentrifuge(s) (with rotor for 1.5 ml and 2 ml centrifuge tubes).
5. Vortexer.

Preparation Before Use

The samples should be processed in strict accordance with the operating instructions attached to the Viral Nucleic Acid Purification Kit. If the extracted viral DNA cannot be used immediately, please store it at -20℃.

Note

1. Completely thew the reagent at room temperature before use, and invert to mix well, spin down the reagent to the bottom of the tube.
2. When preparing PCR reaction solution, all the reagents should be placed on ice and avoid strong light exposure. It is recommended to set at least the following experimental areas and physically isolate them from the preparation of reaction solution to the addition of test sample.
   Area 1: Preparation and distribution of reaction solution.
   Area 2: Preparation of DNA from test samples.
   Area 3: Add the DNA of the test sample to the reaction solution for reaction and detection (**the PCR tube after amplification is forbidden to open!**).

Protocol

1. Each test reaction system is formulated as follows.

Reagent	ASFV (African Swine Fever Virus) PCR Main Reaction Solution	Taq DNA polymerase Mixture
Dosage (μl)	34	1

2. According to the sample size to be detected, calculate the amount of each reagent component, add it into 1.5 ml centrifuge tube, thoroughly mix it, centrifuge it briefly, add 35 μl PCR solution into the set n (n= number of samples +1 tube of positive control +1 tube of negative control) PCR tube, and transfer it to the sampling area (note that due to the error of pipette, the mixed n samples PCR solution is only enough to be divided into n-1 PCR tubes, it is recommended to add one tube when calculating the number of samples for detection).

For example: if 5 samples need to be detected, the reagent should be prepared according to the amount of 8 (n+1) PCR solution, that is: ASFV (African swine Fever virus) PCR main reaction solution 34×8=272 μl, and then add Taq DNA polymerase 1×8=8 μl, and mix the PCR solution into 7 PCR tubes according to each tube 35 μl. Discard the excess PCR solution.

3. Adding Samples

The prepared DNA solution and the control product (usually 1 negative control tube and 1 positive control tube) were added to the PCR tube respectively, 5 μl each, tightly close the tube, spin down the reaction tube, place the reaction tube in the fluorescent PCR detector, and record the sequence of sample placement.

4. PCR reaction:

Steps	Number of Cycles	Temperature (C)	Reaction time (min:sec)
1	1	95	01:00
2	40	95	00:15
		60	00:35

The collection of fluorescence signal (Detector) is set as FAM, the reference (Passive) is set as None, and the data collection is set at 60℃.

* Note: This product does not contain Rox Reference. If the instrument used needs to use Rox as a reference or want to obtain better curve effect, you can order 50×ROX reference Dye (Simgen. Cat. No. 7709005) separately.

Analysis of results:

1. Threshold setting: The threshold is set to 500 (ABI, Roche, Bio-Rad, Agilent Technologies and other imported instruments do not need to set).

2. Quality control standard

2.1. The lower limit for detection of ASF virus DNA is 0.0001 ng/μl.

2.2. Negative control: Ct value > 38 or no Ct value, line shape is straight or slightly oblique, no exponential growth period.

Positive control: typical amplification curve with Ct value ≤35.

3. Sample determination:

3.1. The Ct value of the specimen test result ≤35 or has a significant exponential growth period, which can be directly judged as "detection of ASFV DNA components".

3.2. If the Ct value of the specimen test result is in the range of 35~38, the specimen should be tested repeatedly. If the Ct value of the repeated experiment result is still in the range of 35~38 and there is an obvious exponential growth period, it will be judged to be positive, otherwise it will be negative.

3.3 If the Ct value of the specimen test result is > 38 or no Ct value, it is determined that “ASFV DNA is not detected”.