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Lieferung & Zahlungsart

siehe unsere Liefer- und Versandbedingungen

Zuschläge

- Mindermengenzuschlag
- Trockeneiszuschlag
- Gefahrgutzuschlag
- Expressversand

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（**FOR RESEARCH USE ONLY. DO NOT USE IT IN CLINICAL DIAGNOSTICS !**）

African Swine Fever Virus Nucleic Acid Fluorescence Kit (RT-PCR)

Catalog No: E-AD-P002
50/100/250T

This manual must be read attentively and completely before using this product.

If you have any problems, please contact our Technical Service Center for help (info in the header of each page).

Toll-free: 1-888-852-8623 Tel: 1-832-243-6086 Fax: 1-832-243-6017
Email: techsupport@elabscience.com
Website: www.vetassay-elab.com

Please refer to specific expiry date from label on the side of box.

Please kindly provide us with the lot number (on the outside of the box) of the kit for more efficient service.

Test principle

The African Swine Fever Virus nucleic acid test kit is performed on the applicable instrument to detect DNA extracted from tissue and liquid sample (blood, pathological secretions, saliva, etc). It utilizes the African Swine Fever Virus conserved sequence of VP72, CD2V and MGF gene as multiplex real time PCR amplification target regions.

The fluorescent quantitative PCR instrument can automatically draw a real-time amplification curve based on the detected fluorescent signal, so as to realize the qualitative detection of the ASFV at the nucleic acid level. The multiplex real time PCR detection system includes a pair of primers and probes for internal control. The positive control and negative control included in the kit can be used as an external control in every test run. The results of internal and external control can be used to monitor correct specimen collection, handling and real time PCR process.

Intended use

The African Swine Fever Virus nucleic acid Test Kit is a real-time polymerase chain reaction (PCR) test intended for the qualitative detection of african swine fever virus in tissue and liquid sample (blood, pathological secretions, saliva, etc).

Kit components

The kit should be stored at -20 protected from light until the expiration date printed on the pouch. Repeated freezing and thawing (≥10 cycles) should be avoided.

Item	Specifications	
PCR reaction buffer	1.2/2.4/6 mL	E-AD-P002
Positive Control	0.2/0.4/1.0 mL	E-AD-P002
Negative Control	0.2/0.4/1.0 mL	E-AD-P002
Manual	1 copy	E-AD-P002

Note: Do not mix the components from different batches of kits.

Materials needed but not provided

- Biosafety cabinet
- Personal protective equipment (PPE)
- General laboratory equipment (e.g.tube racks)
- Real time PCR system
- Nucleic acid extraction kit and instrument
- Pulse centrifuge
- Vortex mixer
- Real time PCR reaction tubes (0.2 mL)

- Ice-container
- Transfer pipettes (0.5 μL-1000 μL)
- Sterile tips for transfer pipettes
- Sterile tubes
- Biohazard waste container
- Refrigerator and freezer

Sample collection and preparation

1. Collecting Specimen

Take samples according to conventional methods.

2. Specimen Storage

The collected specimen should be tested as soon as possible. If it is not tested immediately, the samples to be tested should be stored at 2-8 ℃ for no more than 24 hours; at -20 ℃ for no more than three months, below -70 ℃, they can be stored for a long time.

Note: Inappropriate storage of specimens as well as frequent thawing and re- freezing can damage the specimens and should be avoided; otherwise false negative results may occur.

Assay procedure

Note: All work areas should be in separate (Reagent storage and preparation area, Specimen preparation area and Amplification area).

All necessary safety precautions should be taken according to the laboratory guidelines. Precautions must also be taken to prevent cross-contamination of specimens.

1. Reagent Storage and Preparation Area

1.1 Thaw the kit components at 25℃.

1.2 Thaw the **PCR reaction buffer** at room temperature, fully thaw, shake and mix well, centrifuge at low speed for a few seconds, and then divide the solution into PCR reaction tubes (20μL/tube) according to the number of samples to be amplified (sufficient for the number of patient samples and controls). Cap the PCR reaction tubes with care, ensure that the caps are placed correctly, and transfer to the specimen preparation area, store in a refrigerator at 4 ℃ away from light.

Note: It is important to thoroughly vortex the reagents. It is recommended that always taking account a surplus of 10%.

2. Specimens Preparation Area

2.1 DNA extraction

DNA is extracted according to nucleic acid extraction kit (E-AD-P002 does not include components) manual for nucleic acid detection by PCR.

3. Addition of samples

3.1 Pipette 5 μL of each purified DNA sample, negative control and positive control into the corresponding PCR reaction tube containing the PCR reaction buffer.
Note: Change the pipette tip with every step.

3.2 Cap the PCR reaction tubes with care; ensure that the caps are placed correctly.

3.3 Vortex the PCR reaction tubes briefly and centrifuge to collect the solutions at the bottom.

4. Amplification Area

4.1 Settings of detection fluorescence

Channel	Target Gene
FAM	VP72
VIC	$CD2_V$
ROX	MGF

4.2 Put the reaction tubes on a PCR instrument, set up and run the following cycling protocol.

Note: Reaction volume was set at 25μL				
Step		Cycles	T (℃)	Time
Step 1	Initial denaturation	1	50℃	2 min
Step 2	Denaturation	1	95℃	3 min
Step 3	Annealing, extension and testing	45	95℃	10s
			58℃	20s

a) Start the PCR cycler according to its user manual.

Quality control

1. Negative control: no obvious S-shaped amplification curve, and Ct value is shown as Undet.
2. Positive control: three channels have typical S-shaped positive amplification curves, and the Ct value ≤35.

Note: The above two conditions must be satisfied at the same time, otherwise, this test is invalid.

Performance characteristics

1. Limit of Detection (LOD)

Components	LOD
African Swine Fever Virus	8×10^3 copies/μL

Interpretation of test results

On the premise that the experiment is valid, the test results are judged according to Table below.

Table.1 Ct value of each fluorescence channel and judgment of negative and positive results

FAM(VP72)	**VIC(**$CD2_V$)	**ROX(**MGF)	**Results**
There is a clear exponential increase, with a typical S-shaped curve and the Ct value ≤40.			Nucleic acid of African swine fever virus wild strain was detected
Ct value ≤40	Ct value ≤40	Ct value is shown as Undet	The sample detected the African swine fever MGF gene deletion vaccine strain nucleic acid
Ct value ≤40	Ct value is shown as Undet	Ct value is shown as Undet	The sample detected the African swine fever VP72 and CD2V gene deletion vaccine strain nucleic acid
40< Ct value <45	Ct value≤45 or Ct value is shown as Undet	Ct value≤45 or Ct value is shown as Undet	The results are uncertain and require retesting of the sample. If FAM (VP72) displays 40<Ct value<45 (1) VIC (CD2V) and ROX (MGF) showed Ct value ≤ 45, and it was determined that the nucleic acid of African swine fever virus wild strain was detected in the sample; (2) VIC (CD2V) shows a Ct value ≤ 45, while ROX (MGF) shows no Ct value. It is determined that the nucleic acid of the African swine fever MGF gene deletion vaccine strain has been detected in the sample; (3) ROX (MGF) and VIC (CD2V) showed no Ct value. It was judged that the nucleic acid of African swine fever virus CD2V and MGF gene deletion vaccine strains was detected in the samples.
Ct value is shown as Undet	Ct value is shown as Undet	Ct value is shown as Undet	Negative

Limitations

1. As with any molecular test, mutations within the target regions of this test kit could affect primer and/or probe binding resulting in a failure to detect the presence of a virus.
2. False negative results may occur if inadequate numbers of organisms are present in the specimen.
3. The test result dose not rule out the presence of other co-infection pathogens.

Note

1. This product is for scientific research use only.
2. Please read the instructions for use carefully before use, and strictly follow the instructions.
3. Do not interchange components in different batches of kits.
4. Do not use expired products or products with a broken aluminum foil.
5. The specimens must be treated as potential sources of infection, and must be performed using proper PPE against biological risk according to published guidelines and local regulations.
6. Avoid the liquid in contact with eyes and skin. If it splashes onto the skin or eyes, please wash immediately with plenty of water.
7. Cap the reagents immediately after use.
8. All kit components shall be completely thawed before use.
9. The master mix must be well mixed and placed in the ice-container.
10. Use separate pipette tip for each specimen to avoid cross-contamination of specimens which could cause erroneous results.
11. Please pay attention to the note of the biosafety cabinet during the operation procedures.
12. The test accuracy is affected by the specimen collection, storage and transport process.
13. Operate in the biosafety cabinet with clean disinfection and ultraviolet sterilization to prevent the outflow of aerosol and avoid harmful substances entering the respiratory tract.
14. Perform the test in partitions (Reagent storage and preparation area, Specimen preparation area and Amplification area) and prohibit cross-movement of personnel or equipment between areas.
15. Follow the standard biosafety guidelines for handling and disposal of potentially infective material.
16. If you have any questions or suggestions during use, please do not hesitate to contact the manufacturer.
17. Each reagent is optimized for use in the E-AD-P002. Do not substitute reagents from any other manufacturer into the test kit. Do not combine reagents from other E-AD-P002 with different lot numbers.

Storage and expiry date

Storage: Store at -20℃. Must be frozen.

Expiry date: expiration date is on the packing box.