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

## **Swine Streptococcus Suis Universal Detection Kit (PCR)**

Catalog No: E-AD-P003

50T/100T/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](mailto:techsupport@elabscience.com)

Website: [www.vetassay-elab.com](http://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 swine streptococcus suis nucleic acid test kit is performed on the applicable instrument to detect DNA extracted from tissue samples (Lesioned swine lung, tonsil, lymph nodes and spleen), and animal samples infected and dead from lesions (stool, spleen lungs, serum and pleural fluid). The sample processing method should be carried out according to the instructions of the nucleic acid extraction kit. 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 SS 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 swine streptococcus suis nucleic acid Test Kit is a real-time polymerase chain reaction (PCR) test intended for the qualitative detection of swine Streptococcus in tissue samples (Lesioned swine lung, tonsil, lymph nodes and spleen), and animal samples infected and dead from lesions (stool, spleen lungs, serum and pleural fluid).

## Kit components

The kit should be stored at -20°C protected from light until the expiration date printed on the pouch. Repeated freezing and thawing should be avoided.

Item	Specifications	
Taq enzyme	30 µL	E-AD-P003
PCR reaction buffer	1 mL	E-AD-P003
Positive Control	150 µL	E-AD-P003
Negative Control	150 µL	E-AD-P003
Manual	1 copy	E-AD-P003

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

## Storage and expiry date

**Storage:** Store at -20°C. Must be frozen.

**Expiry date:** expiration date is on the packing box.

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**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. Each PCR reaction should have a negative and positive control set to eliminate operational errors.
16. Follow the standard biosafety guidelines for handling and disposal of potentially infective material.
17. If you have any questions or suggestions during use, please do not hesitate to contact the manufacturer.
18. Each reagent is optimized for use in the E-AD-P003. Do not substitute reagents from any other manufacturer into the test kit. Do not combine reagents from other E-AD-P003 with different lot numbers.
19. Before and after the test, the console, Pipette, Pulse centrifuge and other instruments shall be exposed to 1% Hypochlorous acid, 75% ethanol or ultraviolet light for disinfection and sterilization. The test personnel shall wear disposable powder free latex gloves.

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## 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 -80°C to avoid repeated freeze-thaw.

**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 Restore all reagents and samples to room temperature (25°C) to thaw thoroughly before use. All the reagents should be mixed thoroughly by gently swirling before pipetting.

**Note:** All reagents volume is very small and all reagents must be centrifuged before use to remove any liquid adhering to the tube wall.

- 1.2 Calculate the number of reactions (N) required for this experiment, and calculate various test doses required for this experiment according to the reaction system shown in the table below.

**N=Negative control number (1 tube) + Positive control number (1 tube) + Error number (1 tube, necessary) + Sample number**

Item	Final volume
PCR reaction buffer	20 $\mu$ L $\times$ N tube
Taq enzyme	0.5 $\mu$ L $\times$ N tube

**Note: Usually, one tube of liquid is set for each negative and positive control as the result judgment. If necessary, the number of negative and positive tubes can be increased, and the total volume of added reagents will increase, corresponding to the number of reactions (N).**

- 1.3 The above reagents (**PCR reaction buffer** and **Taq enzyme**) by step 1.2 were added into the sterile tube, thoroughly mixed and then centrifuged instantaneously. The mixed reagent was packed into the **PCR reaction tube** according to **20  $\mu$ L/tube**.

## 2. Specimens Preparation Area

### 2.1 DNA/RNA extraction

DNA/RNA is extracted according to nucleic acid extraction kit (E-AD-P003 does not include components) manual for nucleic acid detection by PCR. Negative/positive control are not required to extract DNA or RNA.

## 3. Addition of samples

- 3.1 Take 5  $\mu$ L of **nucleic acid sample**, **Negative control** and **Positive control** into the corresponding 20  $\mu$ L mixed reagent of **PCR reaction tube** (containing the PCR reaction buffer and Taq enzyme). Finally, the volume of the PCR reaction tube is 25 $\mu$ L.

**Note:** Change the pipette tip with every step.

- 3.2 Cap the PCR reaction tubes with care, make sure the cap is tight to avoid leakage.  
3.3 Centrifuge at low speed for 5 s.

#### 4. Amplification Area

4.1 Put the PCR reaction tubes on a PCR instrument, record the placing sequence, and set up and run the following cycling protocol.

Note: Start the PCR cycler according to its user manual. In addition, the ABI series fluorescence PCR instrument does not select ROX correction when setting, and selects None when setting the quenching group.

Note: Reaction volume was set at 25µL(Channel: FAM)					
Step		Cycles	T (°C)	Time	Collect fluorescence signal
Step 1	Pre-denaturation	1	95°C	3 min	No
Step 2	PCR amplification	45	95°C	10 s	No
			60°C	30 s	Yes

#### Quality control

1. Negative control: no obvious S-shaped amplification curve, linear or slightly oblique line, and Ct value is shown as Undet.
2. Positive control: channels have typical S-shaped positive amplification curves, and the Ct value  $\leq 32$ .

#### 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	Results
Ct value < 38	Positive: Streptococcus suis nucleic acid was detected in the sample
$38 \leq \text{Ct value} \leq 42$	The results are uncertain and require retesting of the sample. If the Ct value of the repeated test result is still within the range of 38~42, and there is an obvious Exponential growth trend, it is determined as positive, otherwise it is negative.
Ct value is shown as Undet	Negative: There is no Ct value and no characteristic amplification curve.

#### 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 does not rule out the presence of other co-infection pathogens.
4. **LOD:** 1000 copies/µL.