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Streptococcus suis Type 2 (SS-2) Nucleic Acid Detection Kit (Fluorescent PCR Method)

Product Number: DTK090

Shipping and Storage

1. -20℃±5℃, stored in the dark, transported, and subjected to repeated freeze-thaw cycles no more than 5 times, with a validity period of 12 months.
2. The collected or processed samples should be stored at 2℃~8℃ for no more than 24 hours; If long-term storage is required, it should be stored at -70℃ or below, with no more than 3 freeze-thaw cycles.

Component

Component	50T
SS-2 reaction solution	500μL×2
Enzyme solution	50μL
SS-2 positive quality control product	250μL
Negative quality control product	250μL

Note: Different batches of reagents cannot be mixed.

Description

Streptococcus suis is an important zoonotic pathogen. Based on the diversity of its capsule polysaccharides, Streptococcus suis is divided into 35 serotypes. Streptococcus suis type 2 (SS-2) has the strongest pathogenicity and is also the most common pathogenic serotype in China. This kit is suitable for detecting Streptococcus suis type 2 in samples such as tonsils, organs or muscles, throat swabs, etc., and is used for auxiliary diagnosis of Streptococcus suis type 2 infection.

Application

Streptococcus suis is an important zoonotic pathogen. Based on the diversity of its capsule polysaccharides, Streptococcus suis is divided into 35 serotypes. Streptococcus suis type 2 (SS-2) has the strongest pathogenicity and is also the most common pathogenic serotype in China. This kit is suitable for detecting Streptococcus suis type 2 in samples such as tonsils, organs or muscles, throat swabs, etc., and is used for auxiliary diagnosis of Streptococcus suis type 2 infection.

Applicable instruments

ABI7500, Agilent MX3000P/3005P, LightCycler, Bio-Rad, Eppendorf and other series of fluorescence quantitative PCR detectors.

Specimen collection

Collection of tonsil, visceral or muscle samples: Use sterile scissors and tweezers to cut 1.0g of the sample to be tested, grind it thoroughly in a mortar, add 5mL PBS and mix well, then transfer the tissue suspension into a sterile centrifuge tube and number it for later use.

Pig swab sample collection: Throat swab sampling. During sampling, the swab should be scraped back and forth 3-5 times deep into the throat and upper jaw to collect throat secretions.

Protocol

1. **Sample processing (sample processing area)**
   - **1.1. Sample pre-processing**

Tonsil, visceral or muscle samples: Use sterile scissors and tweezers to cut 1.0g of the sample to be tested, grind it thoroughly in a mortar, add 5mL PBS and mix well, then transfer the tissue suspension into a sterile centrifuge tube and number it for later use. Pig swab sample: throat swab sampling. When sampling, the swab should be scraped back and forth 3-5 times deep into the throat and upper jaw, and the throat secretion should be taken for later use.

1.2. Nucleic acid extraction

We recommend using our company's nucleic acid extraction or purification reagents (magnetic bead method or centrifugal column method) for nucleic acid extraction. Please follow the reagent instructions for operation.

2. Reagent preparation (reagent preparation area)

Based on the total number of samples to be tested, the required number of PCR reaction tubes is N (N=number of samples+1 negative control tube+1 positive control tube); For every 10 samples, an additional 1 sample is prepared. The preparation of each test reaction system is shown in the table below:

reagent	SS-2 reaction solution	Enzyme solution
Dosage (sample size N)	19μL	1μL

Transfer the mixed test reaction solution into a PCR reaction tube at a concentration of 20uL per tube.

3. Sample addition (sample processing area)

Take 5μL of the nucleic acid, positive control sample, and negative control sample extracted in step 1, and add them to the corresponding reaction tubes. Cover the tubes, mix well, and briefly centrifuge.

4. PCR amplification (nucleic acid amplification zone)

4.1. Place the reaction tube to be tested in the reaction tank of the fluorescence quantitative PCR instrument;

4.2. Set the channel and sample information, and set the reaction system to 25μL;

Fluorescence channel selection: Detection channel (Reporter Dye) FAM, Quenching channel (Quencher Dye) NONE, ABI series instruments. Do not select ROX reference fluorescence, select None.

4.3. Recommended loop parameter settings:

step	Cycles	Temperature	Time	Collect fluorescence signals
1	1 cycle	95℃	2min	No
2	45 cycles	95℃	15sec	No
		60℃	30sec	Yes

5. Result analysis and judgment

5.1. Result Analysis Condition Setting

After the reaction is completed, the results will be automatically saved. Based on the analyzed image, adjust the Start value, End~value, and Threshold value of the Baseline (~can be adjusted by the user according to the actual situation, the Start value can be set at 3-15, and the End value can be set at 5-20, so that the threshold line is in the exponential period of the amplification curve, and the amplification curve of the negative quality control product is flat or lower than the threshold line). Click Analyze to automatically obtain the analysis results..

5.2. Result judgment

Positive: The Ct value of the detection channel is ≤ 40, and the curve shows a significant exponential growth curve;

Negative: The sample test result shows no Ct value and no specific amplification curve.

Suspicious: If the sample test result is 40<Ct value ≤ 45, it is recommended to repeat the test. If the detection channel is still 40<Ct value ≤ 45 and the curve has a clear exponential growth curve, it is judged as positive. Otherwise, it is judged as negative.

Quality control standards

1. Negative quality control product: no specific amplification curve or Ct value display;
2. Positive quality control product: The amplification curve shows a significant exponential growth period, and the Ct value is ≤ 32;
3. The above conditions should be met simultaneously, otherwise the experiment will be considered invalid.

Limitations of detection methods

1. The results of sample testing are related to the quality of sample collection, processing, transportation, and preservation;
2. Failure to control cross contamination during sample extraction can result in false positive results;
3. Leakage of positive controls and amplification products can lead to false positive results;
4. During the epidemic, genetic mutations and recombination of pathogens can lead to false negative results;
5. Different extraction methods have differences in extraction efficiency, which can lead to false negative results;
6. Improper transportation, storage, or inaccurate preparation of reagents can lead to a decrease in reagent detection efficiency, resulting in false negatives or inaccurate quantitative testing results;
7. The test results are for reference only. If a diagnosis is required, please combine clinical symptoms and other testing methods.

Note

1. All operations shall be strictly carried out in accordance with the instructions;
2. Before use, all components in the reagent kit should be naturally melted, completely mixed, and briefly centrifuged;
3. The reaction solution should be stored away from light;
4. Try to avoid the presence of bubbles during the reaction and tightly cover the tube cap;
5. Use disposable suction heads, disposable gloves, and specialized work clothes for each area;
6. Sample processing, reagent preparation, and sample addition should be carried out in different areas to avoid cross contamination;
7. After the experiment is completed, treat the workbench and pipette with 10% hypochlorous acid, 75% alcohol, or a UV lamp;
8. All items in the reagent kit should be treated as contaminants and handled in accordance with the "Biosafety Guidelines for Microbial Biomedical Laboratories".