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Porcine Torque Teno Virus Type 1/Type 2 (TTSuV1/TTSuV2) Nucleic Acid Detection Kit (Dual Fluorescent PCR Method)

Product Number:DTK436

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 above specimens can be stored at -20 ℃ in the short term and -70 ℃ in the long term, but cannot exceed 6 months. The specimens should be transported in 2-8 ℃ ice packs, and repeated freezing and thawing are strictly prohibited.

Component

Component	50T
TTSuV1/TTSuV2 reaction solution	500μL×2
Enzyme solution	50μL
TTSuV1/TTSuV2 positive quality control product	250μL
Negative quality control product	250μL

Note: Different batches of reagents cannot be mixed.

Description

Torque Teno Sus (TTSuVs) is a small, non enveloped, single stranded closed circular DNA virus with high genetic diversity. According to nucleotide sequence differences, TTSuV can be divided into two genotypes: TTSuV1 and TTSuV2. TTSuV1 belongs to the Iotatorquevirus genus and has two different genotypes, TTSuV1a and TTSuV1b, while TTSuV2 belongs to the Kappatorquevirus genus and has two different genotypes, TSuVk2a and TTSuVk2b. TTSuV can infect healthy or diseased pig herds. Co infection of TTSuV with circovirus type 2 (PCV2) can cause multiple system wasting syndrome (PMWS) in weaned piglets, and co infection with porcine respiratory and reproductive syndrome virus (PRRSV) can cause dermatitis nephropathy syndrome (PDNS). The pathogenic mechanism is not yet clear.

This kit is suitable for detecting porcine circovirus DNA in specimens such as lymph nodes, spleen, lungs, and serum, and is suitable for auxiliary diagnosis of porcine circovirus infection.

Application

This kit uses porcine circovirus specific primers, combined with specific fluorescent probes, to perform in vitro amplification and detection of porcine circovirus DNA using one-step fluorescent RT-PCR technology, for clinical pathogen diagnosis of suspected infectious materials.

Applicable instruments

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

Specimen collection

Death or slaughter of pigs, collection of lymph nodes, spleen, lungs and other tissues; Collect 5mL of blood from live pigs to be tested; tissue samples: Take lymph nodes, spleen, lungs and other tissues, and place them in sterile centrifuge tubes for future use. Serum sample collection: Use a sterile syringe to extract 5mL of venous blood from the tested pig, place it in a sterile centrifuge tube, centrifuge at 2000r/min~3000r/min for 10 minutes, and transfer the supernatant to a new centrifuge tube for later use.

Protocol

1. Sample processing (sample processing area)

1.1. Sample Preparation

Serum samples: no pre-treatment required, directly used for nucleic acid extraction;

Organizational sample: Take 1g of tissue, cut it into pieces, add 2mL of physiological saline for grinding, and prepare the tissue

Homogenate, centrifuge at 8000r/min for 5 minutes, and take the supernatant for subsequent nucleic acid extraction.

1.2. Nucleic acid extraction

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

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 portions of reaction tubes, an additional 1 portion is prepared. The preparation of each test reaction system is shown in the following table:

reagent	TTSuV1/TTSuV2 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, Quencher Dye NONE, please do not select ROX reference fluorescence for ABI series instruments, 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

(Please refer to the user manuals of each instrument for setting up, taking the ABI7500 instrument as an example)

After the reaction is complete, the results will be automatically saved. Based on the analyzed image, adjust the Start value, End value, and Threshold value of the baseline (users can adjust them according to their actual situation, with Start value set between 3-15 and End value set between 5-20, so that the threshold line is in the exponential period of the amplification curve, and the amplification curve of negative quality control products is flat or below the threshold line). Click Analyze to automatically obtain the analysis results.

5.2. Result judgment

The FAM channel represents the detection result of TTSuV1, while the VIC channel represents the detection result of TTSuV2.

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

Negative quality control product: no specific amplification curve or Ct value display;

Positive quality control product: The amplification curve shows a significant exponential growth period, and the Ct value is ≤32;

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 must be strictly carried out in accordance with the instructions;
2. The various components in the reagent kit should be naturally melted, completely mixed, and briefly centrifuged before use;
3. The reaction solution should be stored away from light;
4. Try to avoid the presence of bubbles during the reaction, and cover the tube tightly;
5. Use disposable suction tips, 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"