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Porcine Circovirus Type 1/Type 2 (PCV-1/PCV-2) Nucleic Acid Detection Kit (Dual Fluorescent PCR Method)

Product Number: DTK420

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
PCV-1/PCV-2 reaction solution	500μL×2
Enzyme solution	50μL
PCV-1/PRV-2 positive quality control samples	50μL
Negative quality control product	250μL

Note: Different batches of reagents cannot be mixed.

Description

This kit is designed with specific primers and probes based on the conserved regions of porcine circovirus type 1 and type 2 genes. Fluorescence PCR technology is used for in vitro amplification and detection of PCV-1 and PCV-2 nucleic acids, which are used for pathogen diagnosis of suspected infected individuals in clinical practice.

Application

Porcine Circovirus is an infectious, congenital tremor, and multi system functional disorder in weaned piglets caused by Porcine Circovirus. The virus has two serotypes, PCV1 and PCV2, PCV1 is non pathogenic. Weaned piglets infected with PCV2 may experience shortness of breath, difficulty, diarrhea, anemia, significant lymphoid tissue lesions, and progressive weight loss.

This kit is used to detect porcine circovirus type 1 and type 2 in samples such as lymph nodes, liver, kidneys, lungs, liver, brain, testes, placenta, and whole blood, and is suitable for auxiliary diagnosis of these two pathogenic infections.

Applicable instruments

ABI series, LightCycler, Bio Rad, Eppendorf and other series of fluorescence quantitative PCR instruments.

Specimen collection

Suspected infected piglets should have their mesenteric lymph nodes or liver removed; Kidney, lung, liver, brain, testis, placenta, lung, lymph nodes and other tissues that have been aborted or stillborn; Collect 5mL of blood from the live pig to be tested using a syringe.

Protocol

1. Sample processing (sample processing area)

1.1. Sample Preparation

Organizational samples: Weigh approximately 1g of the sample, cut and mix it with surgical scissors, and then take 0.5g and grind it in a grinder. Add 1.5mL of physiological saline and continue grinding. After homogenization, transfer it to a 1.5mL sterile centrifuge tube and centrifuge at 8000rpm for 2 minutes. Take 100μL of supernatant and transfer it to a

1.5mL sterile centrifuge tube; Take 100μL of throat swab sample directly into a 1.5mL sterilized centrifuge tube.

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

reagent	PCV- 1/PCV-2 Reaction solution	Enzyme solution
Dosage (sample size N)	20μL	1μL

Transfer the mixed test reaction solution into a PCR reaction tube at a concentration of 21μL per tube.

3. Sample addition (sample processing area)

Take 4μL 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 channels (Reporter Dye) FAM, CY5, 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℃	10min	No
2	40 cycles	94℃	15sec	No
		55℃	30sec	Yes

5. Result analysis and judgment

5.1. Result Analysis Condition Setting

Set Baseline and Threshold: Generally, the analysis is based on the results automatically analyzed by the machine. When the curve shows an overall tilt, adjust the start value (usually within the range of 3-15) and stop value (usually within the range of 5-20) of the Baseline and the Value value of the Threshold (drag the threshold line up and down to be higher than the negative control) based on the analyzed image, and reanalyze the results.

5.2. Result judgment

The FAM channel shows the detection results of porcine circovirus type 1, while the CY5 channel shows the detection results of porcine circovirus type 2.

Positive: The Ct value of the detection channel is ≤ 35, and the curve shows a significant exponential growth curve;

Suspicious: Detection channel 35<Ct value ≤ 38, it is recommended to repeat the test. If the detection channel still has 35<Ct value ≤ 38 and the curve has a clear growth curve, it is judged as positive. Otherwise, it is judged as negative;

Negative: The Ct value of the sample test result is greater than 38 or there is no Ct value.

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".