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

Product Number: DTK088

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, they should be stored at -70℃ or below, with no more than 3 freeze-thaw cycles.

Component

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

Note: Different batches of reagents cannot be mixed.

Description

This kit uses TaqMan probe method for real-time fluorescence PCR technology, designs a pair of porcine circovirus type 2 specific primers, and combines them with a specific probe to amplify and detect the DNA of porcine circovirus type 2 in vitro using fluorescence PCR technology, which is used for pathogen diagnosis of suspected infectious materials 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, with PCV1 being non pathogenic. PCV2 has a strong immunosuppressive effect and is often manifested as a mixed infection of multiple pathogens in clinical practice. PCV2 is closely related to multiple system failure syndrome (PMWS) in weaned piglets and can also cause diseases such as dermatitis and nephritis syndrome, respiratory syndrome, and A2 type congenital tremor in pigs, causing huge economic losses to the pig industry. Therefore, early diagnosis of PCV2 infection is particularly important.

This reagent kit is suitable for detecting porcine circovirus type 2 in dead pigs (including stillbirths caused by miscarriage) or major organs (lymph nodes, spleen, lungs, kidneys, and liver), serum, and other samples of slaughtered pigs. It is used for auxiliary diagnosis of porcine circovirus type 2 infection.

Applicable instruments

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

specimen collection

1. Organizational sample collection: Take dead pigs (including stillbirths caused by miscarriage) or major organs of slaughtered pigs (lymph nodes, spleen, lungs, kidneys, and liver) and put them into sterilized 15mL centrifuge tubes, number them, and send them to the laboratory.
2. Serum sample collection: Use a sterile syringe to extract no less than 5mL of venous blood from the tested pig, place it in a sterile centrifuge tube, and let it naturally aggregate at room temperature or 37℃ for 20 to 30 minutes. Centrifuge at 2000 r/min

to 3000 r/min for 10 minutes, and transfer the supernatant to a new centrifuge tube for later use.

3. Fecal sample collection: Take 1g of fresh pig feces, transfer it into a 15mL sterilized centrifuge tube, add 5mL of PBS, and mix well.
4. Cell culture: The cell culture is repeatedly frozen and thawed three times. After the third thawing, the cell culture is placed in a 1.5mL sterile centrifuge tube without DNA enzyme and numbered for future use.

Protocol

1. Reagent Preparation (Reagent Preparation Area)

1.1. Sample pre-processing

Organizational sample: Take 1g of thawed tissue, cut it into pieces, add 2mL of PBS for grinding, prepare tissue homogenate, centrifuge at 8000 r/min for 5 minutes, and take the supernatant for subsequent nucleic acid extraction. Serum and semen samples do not require pre-treatment and can be directly used for nucleic acid extraction. Fecal sample: Centrifuge at 3000r/min for 10 minutes, take the supernatant, transfer it into a 1.5mL centrifuge tube for subsequent nucleic acid extraction. Cell culture: 4000 r/min, centrifuged at 4℃ for 10min, and the supernatant was taken for subsequent nucleic acid extraction.

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. Sample processing (sample processing 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	PCV-2 reaction solution	Enzyme solution
usage	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

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

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 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".