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Fowlpox Virus (FWPV) Nucleic Acid Detection Kit (Fluorescent PCR Method)

Product Number: DTK549

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
FWPV reaction solution	1000μL
Taq enzyme	30μL
FWPV positive quality control product	1000μL
Negative quality control product	1000μL

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 specific primers for chicken pox virus, and combines with a specific probe [1] to amplify and detect the nucleic acid of chicken pox virus in vitro using fluorescence PCR technology, which is used for pathogenic diagnosis of suspected infectious materials in clinical practice.

Application

Avian Pox is caused by the Fowlpox virus and is an acute, contagious disease of chickens. The disease is characterized by the appearance of papules on the hairless or undercoated skin of chickens, or the formation of cellulose necrotic pseudomembranes in the oral and pharyngeal mucosa. In collective or large-scale chicken farms, it is easy to cause epidemics, which can slow down weight gain and lead to emaciation; When laying hens are infected, their egg production temporarily decreases, which can cause more deaths.

This kit is suitable for the nucleic acid detection of chicken pox virus in throat/cloaca swabs, tissue organs, blood and other samples.

Applicable instruments

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

Specimen collection

Kill suspected infected poultry, collect tissues such as fallopian tubes, uterine mucosa, ovaries, etc., place them in sterile centrifuge tubes for later use, or collect cloacal swabs. Rotate the swabs deeply into the cloacal cavity at least 3 times and collect a small amount of feces.

Protocol

1. Sample processing (sample processing area)

1.1. Sample Preparation

Organizational sample: Take an appropriate amount of tissue sample, grind it, and prepare a suspension by adding 5mL PBS to 1g of tissue. Centrifuge at 3000r/min for 10 minutes, and take the supernatant for subsequent nucleic acid extraction.

Swab sample: Immerse the swab in 5mL PBS, vortex and shake thoroughly for 1 minute, repeatedly squeeze the swab, discard the swab, centrifuge the leachate at 3000r/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)

2.1. System preparation:

Take out the fluorescent PCR reaction solution and Taq enzyme from the reagent kit, melt at room temperature, and centrifuge at 2000 r/min for 5 seconds. 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	FWPV Reaction solution	Taq enzyme
Dosage (sample size N)	19.5μL	0.5μL

2.2. System packaging:

After mixing and centrifuging the above reaction solution, package 20μL per tube into PCR tubes suitable for fluorescence PCR equipment.

2.3. Sample addition:

Add 5μL of corresponding DNA solution to the PCR tubes mentioned above, cover the tubes tightly, and centrifuge at 500 r/min for 30 seconds.

3. Recommended loop parameter settings:

step	Cycles	Temperature	Time	
1	1 cycle	50℃	2min	
2	1 cycle	95℃	3min	
3	40 cycles	95℃	15sec	
		60℃	30sec	Collect fluorescence

4. Result analysis and judgment

4.1. Result analysis condition setting

Threshold setting principle: Adjust according to the instrument noise situation, with the threshold line just exceeding the highest point of the negative control amplification curve as the standard. Select the HEX/VIC detection channel to read the detection results. ABI instrument should choose no fluorescence quenching group, and ROX cannot be selected as the reference group.

4.2. Result judgment

Positive: The Ct value of the detection channel is ≤ 36, and the curve shows a significant exponential growth curve;

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

Suspicious: Samples with Ct values greater than 36 and typical amplification curves are recommended to be retested Test. If the above results still appear during retesting, it will be judged as positive; otherwise, it will be 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".