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Fowl Adenovirus Detection Kit (Real-Time PCR Method)

Product Number:DTK008

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
FADV reaction solution	500μL×2
Enzyme solution	50μL
FADV positive control substance	50μL
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 avian adenovirus specific primers, and combines with a specific probe [1] to amplify and detect the nucleic acid of avian adenovirus in vitro using fluorescence PCR technology, which is used for pathogen diagnosis of suspected infected individuals in clinical practice.

Application

Fowl Adenovirus (FADV) is an enveloped, linear double stranded DNA virus belonging to the family Adenoviridae and genus Adenovirus. This virus can infect chickens, turkeys, and other poultry. FADV can cause diseases such as inclusion body hepatitis, pericardial effusion syndrome, and myogastric erosion in chickens. This disease is often co infected with other pathogens in poultry, and infected chickens can develop secondary conditions such as aplastic anemia, hepatitis, and decreased egg production. FADV has a global distribution and can be horizontally or vertically transmitted through the fecal oral route, causing significant economic losses to the poultry industry.

This kit is suitable for detecting avian adenovirus in samples such as cotton swabs of respiratory secretions (chickens, turkeys, geese, quails infected with Group I avian adenovirus), ovarian, fallopian tube, follicular, uterine and fallopian tube mucosal tissues, and cloacal swabs (chickens, ducks infected with Group II and III avian adenovirus), for auxiliary diagnosis of avian adenovirus infection.

Applicable instruments

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

specimen collection

Extract ovarian, fallopian tube, follicle, uterine, and fallopian tube mucosal tissues from dead poultry; Take respiratory swabs or cloacal swabs from live chickens and place them in 1mL of 50% glycerol physiological saline solution.

Protocol

1. Reagent Preparation (Reagent Preparation Area)

1.1. Sample pre-processing

Organizational sample: Weigh about 1g of the sample, cut and mix it with surgical scissors, 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 the supernatant and transfer it to a 1.5mL sterile centrifuge tube; Take 100μL of cloacal swab directly for 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	FADV 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 Ct value of the sample test result is greater than 40 or there is no Ct value.

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".