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Chicken Infectious Anemia Virus (CIAV) Nucleic Acid Detection Kit (Fluorescent PCR Method)

Product Number: DTK004

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
CIAV reaction solution	500μL×2
Enzyme solution	50μL
CIAV positive quality control product (4.12×10^5copies/mL)	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 chicken infectious anemia virus specific primers, and combines them with a specific probe to amplify and detect the nucleic acid of chicken infectious anemia virus in vitro using fluorescence PCR technology, which is used for pathogenic diagnosis of suspected infectious materials in clinical practice.

Application

This kit is suitable for detecting chicken infectious anemia virus in samples such as thymus and bone marrow, and is used for auxiliary diagnosis of chicken infectious anemia virus infection. The test results are for reference only.

Applicable instruments

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

Specimen collection

CIAV exists in tissues and organs such as the liver, skin, spleen, thymus, bursa of Fabricius, kidneys, and bone marrow of diseased chickens. Aseptically collect the above tissues, prepare a 20% tissue suspension using serum-free DMEM, centrifuge at 3000 r/min for 30 minutes, and collect the supernatant. Other sample collection can refer to SNT4053-2014.

Protocol

1. Sample processing (sample processing area)

1.1. Sample Preparation

Take 25mg of thymus, bone marrow and other tissues from the chicken to be tested, add 4 times the volume of TE, and homogenize; Freeze thaw the homogenized tissue three times, centrifuge at 3000 r/min for 30 minutes, and collect the supernatant.Other sample collection can refer to SNT4053-2014.

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. 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 table below:

reagent	CIAV 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

5.2.1. If this kit is used for qualitative testing:

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: 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 significant growth curve, it is judged as positive. Otherwise, it is considered negative.

5.2.2. If this reagent kit is used for quantitative detection:

Draw a standard curve with the log value of positive control concentration as the horizontal axis and Ct value as the vertical axis. Calculate the log value of the DNA concentration of the sample from the standard curve based on the Ct value of the sample to be tested, and then calculate its concentration.

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".