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Chicken Nucleic Acid Detection Kit (Fluorescent PCR Method)

Product Number: DTK355

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
Chicken reaction solution	500μL×2
Enzyme solution	50μL
Chicken positive quality control product	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 chicken derived gene conserved region specific primers, and combines them with a specific probe to perform in vitro amplification and detection of chicken derived gene conserved region DNA using fluorescence PCR technology, thereby achieving rapid detection; Used for pathogen diagnosis of suspected infectious materials in clinical practice.

Application

Animal derived ingredients are widely distributed in food, feed, cosmetics, etc., and are closely related to people's lives, with food and feed accounting for the largest proportion. The adulteration of meat and meat products has become a hot topic of concern for countries around the world, and the safety hazards of meat products and their feed directly affect human safety. In recent decades, animal derived component detection with PCR technology as the core has been widely applied.

This kit is suitable for the detection of chicken derived components in animal tissues, food, feed and other samples.

Applicable instruments

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

Specimen collection

Take approximately 1g of animal tissue and its products, food or feed.

Protocol

1. Sample processing (sample processing area)

1.1. Sample Preparation

For feed samples, sampling and processing shall be carried out in accordance with GB/T14699.1-2005 Feed Sampling. Take animal tissues, their products, and food. After surgical cutting and mixing, 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 put it in a 1.5mL sterile 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	Chicken Reaction solution	Enzyme solution
Dosage (sample size N)	19μL	1μL

Transfer the mixed test reaction solution into a PCR reaction tube at a concentration of 20μL 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	40 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 ≤ 35, and the curve shows a significant exponential growth curve;

Negative: The Ct value of the sample test result is >35 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.

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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".