Avian Infectious Laryngotracheitis Virus PCR Nucleic Acid Detection Kit

Please read the datasheet carefully before use.

Cat. No. Z-AQ712-DV409-2

Specification: 48 tests/ box

Description

Using Taqman probe technology, specific primers and probes were designed with the gB gene of chicken infectious laryngotracheitis virus (ILTV) as the target site for the auxiliary detection of ILTV nucleic acid in samples. This product introduces a dUTP/UDG system that degrades U-containing ssDNA and dsDNA, eliminating cross-contamination caused by PCR products. At the same time, a positive internal control (that is, an internal standard, using a VIC reporter group) is set to monitor whether the qPCR system contains PCR inhibitors by detecting whether the internal standard is normal to avoid false-negative results.

Kit Contents

Name	Component	Specification
ILTV-PreMix	qPCR reaction buffers, dNTPs, UDG enzymes, Taq enzymes, primers, probes, etc.	960 μl/ tube
ILTV Negative Control	Nuclease-free Water	400 μl/ tube
ILTV Internal Standard Control	Nucleic acid containing amplified fragment of internal standard	480 μl/ tube
ILTV Positive Control	Nucleic acid containing amplified fragment of internal standard	50 μl/ tube

Note: The ingredients of different batches of products cannot be mixed or interchanged.

Storage and Shelf Life

Store in the dark at -20±5°C.

Valid for 12 months.

Shipping with dry ice.

Repeat freeze-thaw less than 5 times. Please refer to the outer box for the production date and expiration date.

Applicable instruments

ABI7500, Hongshi 96P, Bio-RadCFX96 real-time PCR instrument, etc.

Sample Requirements

1. Applicable Sample Types

The laryngo, trachea, muscle or lung tissues are collected from dead or killed birds. For live birds to be tested, respiratory secretions or excreta are collected with cotton swabs and placed in the virus sample preservation solution.

Fresh samples are required to be sent for inspection, and repeated freezing and thawing are prohibited!

2. Sample processing (nucleic acid extraction area)

Sample pretreatment is carried out following "NY/T 541-2016 Technical Specifications for Collection, Preservation and Transportation of Veterinary Diagnostic Samples" and other standards, with a commercial extraction kit used to extract nucleic acids in the samples. During extraction, add 10uL of internal standard control to each 200uL of negative control and processed sample solution. The extracted nucleic acid should be detected immediately, otherwise, it should be frozen at -20±5°C, and the storage time should not exceed 6 months. **Do not repeat freezing and thawing!**

Other methods of using the internal standard control: Mix the internal standard control into the qPCR working solution, but only for quality control of the amplification process and to monitor whether the qPCR system contains PCR inhibitors. It cannot quality control the nucleic acid extraction.

Detection Method

To avoid contamination, the experiment needs to be performed in separate areas!

Note: Nucleic acid extraction is performed in the nucleic acid extraction area or the sample processing area according to the sample requirements.

1. Reagent preparation (in the reagent preparation area)

(1) Take out the components and self-prepared reagents in the kit. Place them at room temperature. Wait for the temperature to equilibrate to room temperature. And mix well for later use;

(2) qPCR working solution preparation (avoid direct light source during the whole process)

Dispense a sufficient amount of PreMix according to the number of samples tested, and it is recommended to set negative and positive controls for each test.

When selecting the **internal standard control quality control nucleic acid extraction and nucleic acid amplification.** When the number of samples to be tested is n, the number of aliquots of qPCR amplification solution N = number of samples to be tested (n) + positive control (1) + negative control (1) + 0.5, then aspirate 20 μl×N (PreMix). It is recommended to use it each time to reduce reagent contamination.

(3) Mix the prepared qPCR working solution thoroughly and centrifuge briefly before use.

(4) When ready to use the working solution in (3), dispense an equal volume of 20 μl into each microcentrifuge tube (eg, eight-tube) and transfer it to the sample processing area.

2. Sample Loading (in the sample processing area)

Add 5 μl of the extracted sample to be tested, negative control and positive control to each PCR tube, cover the tube and centrifuge briefly to make the liquid attach to the bottom of the tube.

3. qPCR amplification (in the amplification and analysis area)

(1) Fluorescence Channel Selection

Selects the **FAM** channel to detect **ILTV**, the **VIC** channel to detect the internal standard, and the quencher to none. As for the ABI qPCR instrument, it also needs to set Passive Reference to none. For other instruments, refer to the instrument manual.

(2) qPCR Amplification Program Settings

Set the reaction volume to 25 μl

Procedure		Temperature	Reaction Time	Cycle
UDG digestion		50°C	5 min	1
Predenaturation		95°C	30 s	1
Amplify	Denaturation	95°C	5 s	45
	Annealing and Extending	60°C	35 s, data collection	

4. Result Analysis (taking the ABI qPCR instrument as an example)

After the reaction is complete, the qPCR instrument will automatically generate results. If the amplification curve or threshold line automatically generated by the instrument is abnormal, the user can adjust it according to the actual situation. The baseline Start value can be set at 3~10, and the baseline End value can be set at 5~20 level. Click Analyze to analyze.

5. Quality Control

Each control in the kit should meet the following requirements, otherwise, the experiment will be invalid.

	Positive Control	Negative Control
FAM channel (IBV)	Ct≦32	1No Ct value or Ct>40
VIC channel (internal standard)	No Ct value or Ct>40	Typical S-shaped curve, Ct ≦35

When the internal standard is mixed with PreMix, the VIC channel should show an S-shaped curve, and the Ct value should be less than or equal to 35.

Interpretation of Test Results

(1) If the VIC channel of the sample shows a typical S-shaped curve and Ct≦35, when the FAM channel Ct≦35, it is determined as positive; when the FAM channel Ct value is between 35 and 40, the amplification curve of the target gene needs to be observed whether it is S-shaped or not. If the curve is S-shaped, the sample should be regarded as suspected positive and need to be re-tested; when the FAM channel Ct>40, it is determined as negative.

(2) If the Ct value of the VIC channel is higher than 35 without showing a clear sigmoid amplification curve, the reasons are as follows:

1) PCR inhibitors are present in the sample. It is recommended to dilute the sample before testing.

2) Nucleic acid extraction operation is defective. It is recommended to repeat nucleic acid extraction for detection.

3) If a qualified sample is not obtained during processing, or the sample has degraded during transportation and storage, re-sampling is recommended.

Limitations of Test Methods

1) Negative results may be due to the low quality of nucleic acid extracted from the sample, improper storage conditions, inappropriate storage period, presence of inhibitors in the sample, nucleic acid degradation and poor extraction efficiency of the nucleic acid extraction method.

2) Unreasonable sample collection, transportation and storage conditions, too low virus content in the sample, or unreasonable experimental operation and environment are likely to cause false-negative or false-positive results. Other clinical observations and related data should be combined with the determination, and the test should be performed again if necessary.

3) Sequence changes in the target sequence, due to mutation or other reasons, may produce false-negative results.

Notes

1) This product is only for scientific research use, please read this manual carefully before use.

2) Before the experiment, please acquaint and master the operation methods and precautions of various instruments to be used, and conduct quality control of each experiment.

3) Laboratory management must strictly follow the management specifications of PCR gene amplification laboratories. The experimental personnel must undergo professional training. The experimental process is strictly carried out by separate areas. All consumables are only for one-time use. Supplies in different areas and stages cannot be used interchangeably.

4) All testing samples are regarded as infectious substances. During the experiment, wear work clothes and change gloves frequently to avoid cross-contamination between samples;

5) Sample handling and waste disposal should comply with local relevant regulations: "General Biosafety Standard for Microbiological and Biomedical Laboratories" and "Medical Waste Management Regulations"

6) All reagents (including the components in this product and customer-supplied reagents) must be thawed and mixed thoroughly before use.

7) Severe hemolysis of serum samples will significantly reduce the height of the amplification curve. Therefore, when using this kit to detect the serum sample stock solution and the normal diluted by saline sample at the same time, it is recommended to set different threshold lines for the two types of templates to ensure the accuracy of the results.

8) When the Ct value of multiple samples is greater than 40, it may be caused by the high threshold line automatically generated by the qPCR instrument. It is recommended to set the threshold line height at about 1/15 of the maximum fluorescence value of the amplification curve before re-analysis.

This product is for scientific research use only, and the test results cannot be used as the sole basis for clinical diagnosis and judgment.

FOR RESEARCH USE ONLY