

Avian Influenza Virus H9 Subtype Nucleic Acid Detection Kit (Fluorescence PCR Method) User Guide



P939H

FOR ANIMAL USE ONLY!

Version 1.0

For use with qPCR Instruments compatible with Avian Influenza Virus H9 Subtype
Nucleic Acid Detection Kit (Fluorescence PCR Method)



P939H



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Introduction

Avian Influenza Virus (AIV) is a member of the Orthomyxoviridae family that primarily infects birds. This virus can cause acute respiratory diseases in avian species, and severe cases often result in high mortality rates. AIV is categorized into subtypes based on two surface proteins, namely hemagglutinin (H, such as H5, H7, H9) and neuraminidase (N, such as N1, N2). The H9 subtype of AIV (primarily H9N2 subtype), classified as low pathogenic avian influenza, has become endemic in poultry populations across Asia, the Middle East, and Africa. While typically causing mild respiratory signs and reduced egg production in birds, H9N2 poses significant concerns due to its dual role as both a frequent gene donor for dangerous reassortant viruses (contributing internal genes to H5N1, H7N9 and other subtypes) and its demonstrated zoonotic potential. Therefore, rapid and accurate diagnosis of H9 is crucial. The **Avian Influenza Virus H9 Subtype Nucleic Acid Detection Kit** developed by Tianlong assists in the diagnosis of AIV H9 Subtype and public health management.

Intended Use

The **Avian Influenza Virus H9 Subtype Nucleic Acid Detection Kit** of Tianlong is intended for the qualitative detection of AIV-H9 RNA by Reverse Transcription Quantitative Real-Time Polymerase Chain Reaction (RT-qPCR) method.

The test is designed to detect RNA from AIV-H9 in specimens such as poultry tissue organs, serum, feces, cloaca, and throat swab samples.

The test results are for veterinary clinical reference only and cannot be used as the sole basis for confirming or excluding cases.

The Tianlong **Avian Influenza Virus H9 Subtype Nucleic Acid Detection Kit** is to be used with qPCR instruments with 2 or more fluorescence detection channels. The kit's performance has been validated on such real-time PCR systems, which include compatible fluorescence channels for FAM and VIC, e.g. Tianlong Gentier Real-time PCR system, Applied Biosystems™ QuantStudio™ 5 Real-Time PCR System and Applied Biosystems™ 7500 Real-Time PCR System.

Kits Components

Ref no.		P939H
Number of reactions		50T
RT-qPCR reagents		
AIV-H9 Fluorescence Reaction Buffer	950 µL	1 tube
AIV-H9 Enzyme Mixture	50 µL	1 tube
Controls		
AIV-H9 Positive Control	50 µL	1 tube
AIV-H9 Negative Control	50 µL	1 tube
AIV-H9 Internal Control	250 µL	1 tube

Table 1: Kits components

Note: All reagents should be stored at -25°C to -15°C in a non-frost-free freezer. Do not mix the reagents from different batches. The negative control can be used as a “No Target Control” (NTC).

Materials Required but Not Provided

- Microvolume pipettes* dedicated for PCR (0.1-2.5 µL; 1-10 or 1-20 µL; 20-200 µL; 1000 µL).
- Benchtop centrifuge* with rotors for 0.5 mL/1.5 mL reaction tubes (capable of attaining 10,000 rpm).
- Benchtop vortex mixer*.
- It is recommended to use this detection kit in qPCR instruments with appropriate fluorescence channels for FAM, VIC dyes such as Applied Biosystems™ QuantStudio™ 5 Real-Time PCR System, Applied Biosystems™ 7500 Real-Time PCR Systems and Tianlong Gentier Real-time PCR system.

(*): Ensure that instruments have been checked and calibrated according to the manufacturer’s recommendations.

Note: please refer to the corresponding manufacturer’s Instructions for Use (IFU) to operate the instrument.

Principles of the Assay

The kit is designed with specific primers and probes targeting Avian Influenza Virus H9 Subtype (AIV-H9) conserved gene segment. The probe is designed to bind specifically to a section of complementary DNA (cDNA) within primer amplification area. During PCR extension reaction process, the exonuclease activity of Taq enzyme will cleave 5'-end fluorophore from the probe to make it free in reaction system and break away from shielding of 3'-end fluorescence quencher, which allows the fluorophore to accept the optical excitation, emit fluorescence for instrument testing and achieve automatic testing for AIV-H9 nucleic acid by this way.

In this kit, a synthetic non-competitive sequence was designed as an internal control, which did not interfere with the target gene. The specific primers and probes were designed according to this internal control. VIC channel was selected for internal control to monitor the testing process and effectively detect the occurrence of false negative results.

Reagent Storage and Handling

All reagents should be stored at -25°C to -15°C for a maximum of 12 months. The stability of unused reagents is not influenced by re-storage. But the number of times of freeze-thaw cycles should not be more than three times. The opened reagents should be kept no more than 8 hours at room temperature. The products should be shipped in an ice box or a refrigerated truck between 2°C and 8°C. Simulated transport tests indicate that the stability and validity of the reagents are not influenced by transport.

Sample Requirements

The **Avian Influenza Virus H9 Subtype Nucleic Acid Detection Kit** is designed to detect Avian Influenza Virus-H9 RNA in poultry tissue organs, serum, feces, cloaca and throat swab samples.

Storage: samples can be stored at 2-8°C for a maximum of 24 hours; at -20°C for a maximum of 3 months, while repeated freeze-thaw cycles should be avoided.

Transportation: samples should be transported in a sealed foam box with ice.

Extraction of nucleic acid should be carried out according to the reagent manufacturer's instructions.

Before Starting

- Identify the product.
- Verify the expiration date.
- Verify the latest instruction for use available for the product lot number.
- Check if the product has been used previously. If so, check the remaining tests available.

Nucleic Acid Extraction

Tianlong **Avian Influenza Virus H9 Subtype Nucleic Acid Detection Kit** is compatible with high-quality RNA/nucleic acids prepared from intended samples using common RNA/nucleic acid extraction kits or methods. The prepared RNA/nucleic acids can be used directly as sample RNA/nucleic acid material and can proceed directly to the real-time PCR reaction setup step.

We recommend adding 5 µL AIV-H9 Internal Control to 200-400 µL sample and extracting AIV-H9 and the internal control together when extracting nucleic acid from samples (For Tianlong Animal Virus DNA and RNA Extraction Kit Series).

- 1) Serum, feces, cloaca and throat swab samples: extract nucleic acids directly according to the instructions of the nucleic acid extraction kit.
- 2) Tissue sample treatment: weigh about 1 g of each tissue sample from three different locations, and cut them into pieces with surgical scissors and mix well; then, put 0.05-0.15 g of the tissue sample into the grinder, add 1.5 mL of normal saline and continue to grind it thoroughly; after homogenization, transfer it to a 1.5 mL sterilized centrifuge tube, centrifuge it at 12,000 rpm for 5 min, take 400 µL supernatant for nucleic acid extraction.

Positive Control and **Negative Control** do not need to be extracted and can be tested directly in each RT-qPCR assay run. If prepared RNA/nucleic acids need to be stored frozen for later testing, storage at -70°C or lower, whenever possible, is recommended for minimal nucleic acid degradation during storage. Repeated freeze-thaw cycles of prepared RNA/nucleic acids should be avoided whenever possible.

RT-qPCR Reaction Setup

1. Thaw the following reagents on ice: **AIV-H9 Fluorescence Reaction Buffer** and **AIV-H9 Enzyme Mixture**. Gently and evenly mix each individual reagent, then briefly centrifuge the reagents (2000rpm, 10sec) to collect the contents.
2. Set up a premix solution based on the number of sample preparations to be tested. The volume of the premix required for all sample preparations to be tested = (the number of samples + 2 controls) * the total volume of premix reagents (as listed in Table 2).

Premix reagents	Volume
AIV-H9 Fluorescence Reaction Buffer	19 µL
AIV-H9 Enzyme Mixture	1 µL
Total volume	20 µL

Table 2 : Premix reagents

3. Evenly aliquot 20 µL of the premix reagents into each PCR tube (one tube per test sample). Add 5 µL of each extracted RNA solution to a single PCR tube. Do not add more than one sample of extracted RNA into a single PCR tube. Add 5 µL of **AIV-H9 Positive Control** and **AIV-H9 Negative Control** into two separate qPCR tubes, respectively. **Each PCR tube should have a total volume of 25 µL. Then immediately close the tubes and transfer the reaction setup into a qPCR machine for amplification.**

Thermal Cycler Settings

Set up the following thermal cycling program. It is recommended to use a qPCR system with 2 or more fluorescence detection channels.

Stage	Number of Cycles	Temperature (°C)	Time (min: sec)
1	1	50	10:00
2	1	95	03:00
3	45	95	00:15
		60	00:30 (collect fluorescence)
4	1	25	00:10

Table 3 : qPCR cycling program

Selection of fluorescence channels: AIV-H9 (FAM) and Internal Control (VIC).

Detection Channels

Two channels are used in this one-tube qPCR assay. It is recommended to perform the optical (channel) calibration as requested by the instrument's manufacturer. Please refer to the instrument's user manual to perform the optical (channel) calibration. Assign appropriate fluorescence channels for each target when using Tianlong's **Avian Influenza Virus H9 Subtype Nucleic Acid Detection Kit**.

Threshold Value Setting Principle:

- Manual setting: set the threshold value slightly above the maximum fluorescence value observed in the negative control amplification curve.
- Auto setting: the instrument automatically sets the threshold value.

Result Analysis



1. Negative control: there is no typical S-shaped amplification curve in FAM.
2. Positive control: there are typical S-shaped amplification curves and the Ct values in both FAM and VIC channels are ≤ 30 .
3. The internal control Ct value of the test samples should be ≤ 35 . If there is no Ct value in the internal control for the test sample, please find out the reasons and retest the sample.
4. The test is effective if conditions 1, 2 and 3 are satisfied simultaneously; otherwise, it is invalid.

Result Interpretation:

After the above quality control conditions are met, carry out the following analysis (the AIV-H9 is detected in the FAM channel and the internal control is detected in the VIC channel):

FAM (AIV-H9)	VIC (Internal Control)	Result
Ct <45	Ct ≤ 35	AIV-H9 Positive
No Ct value	Ct ≤ 35	AIV-H9 Negative
/	No Ct value or Ct >35	Invalid test and need to be checked and retested.

Table 4: Result interpretation

Performance Characteristics

The following performance characteristics of the Tianlong's **Avian Influenza Virus H9 Subtype Nucleic Acid Detection Kit** have been established following the procedure described in this datasheet.

Non-clinical studies

- Limit of detection: 1000 copies/mL.
- Specificity: No cross-reactivity was observed with other common pathogens sharing the same infection site or similar infection symptoms.
- Precision: The assay was used to respectively detect the standard reference specimens of high and low concentrations at different time points. Each detection was carried out 20 times respectively, and the precision values of intra-assay and inter-assay Ct values were all <5%.

Limitations

- All reagents in the kit are intended for veterinary and poultry use as specified.
- The test should be carried out by professionals with adequate training in laboratory practices. It is the user's responsibility to verify/validate the testing system performance in their respective laboratory settings. Expired reagents should not be used.
- Strict compliance with the Instructions for Use (IFU) is required for optimal results. Deviation from standard procedures during sample collection, preservation, transportation, processing and testing could lead to false negative or false positive testing results.
- Theoretically, variations in the target sequences of AIV-H9 arising from natural mutations could potentially influence testing performance and result in false testing results. To date, results from bioinformatics analysis and comprehensive laboratory studies show that, because of the emphasis on the mutation - tolerance concept in assay design and development, this kit could tolerate currently known AIV-H9 mutations without significantly compromising assay performance.
- Test results should be combined with clinical and epidemiological information to make medical decisions.

Warnings and Precautions

Laboratory precautions

Use extreme caution to prevent:



- RNase contamination which may cause degradation of the RNA template
- RNA or PCR carryover contamination which may result in false positive signals

We therefore recommend the following:

- To ensure accurate and reliable results, always use DNase/RNase-free disposable pipette tips, tubes and calibration pipettes.
- Use separate working areas: 1) reagent preparation area- preparing the reagents for amplification, 2) sample preparation area- isolating the RNA/DNA from samples and controls, and 3) amplification area- amplifying and detecting the target nucleic acid.
- To avoid contamination, all the objects should be used in certain areas. All apparatus must be cleaned after each experiment.
- To avoid the contamination of fluorescence materials, disposable gloves, tubes, pipettes and filter tips should not contain fluorescence materials.
- Avoid generating bubbles when transferring the reaction solution into each tube separately. Check the tubes before amplification to avoid contamination induced by leakage of fluorescence materials.
- Nucleic acid samples stored at -70 ° C should be thawed, mixed and centrifuged at low temperature for a short time before use.
- The reaction tube containing the reaction solution should be capped or packed in a sealed bag and then transferred to the sample processing area.
- When adding the sample, the sample should be completely added to the reaction solution, and no sample should adhere to the tube wall. The tube cap should be closed as soon as possible after the sample is added.
- When dispensing the reaction solution, try to avoid generating air bubbles, and check whether the reaction tubes are tightly closed before loading into the machine to avoid the leakage from contaminating the instrument.
- After the amplification, the reaction tube should be taken out, sealed in a special plastic bag, and discarded at the designated place.
- The used tips should be thrown into disposable waste bottles containing 10% sodium hypochlorite solution and then be disposed of with other biomedical waste.
- Use 10% sodium hypochlorite, 75% alcohol and ultraviolet light to disinfect the workbench and experimental items regularly.
- The real-time PCR instrument requires frequent calibration and cleaning of the wells of the plate.
- The samples to be tested involved in this kit shall be regarded as infectious substances, and the operation and treatment shall comply with the relevant requirements of the General Guidelines for Biosafety of Microbial Biomedical Laboratories and the Medical Waste Management Regulations issued by the Ministry of Health.

Safety Symbols and Signs










No.	Symbol	Implication
1		Catalogue number
2		Batch code
3		Contains sufficient for <N> tests
4		Use by date
5		Caution
6		Temperature limit
7		Manufacturer
8		Date of manufacture
9		PAP21: Not-corrugated cardboard

Table 5: Safety symbols and signs

Contact Information

For technical assistance and more information, please contact our Technical Support Center at +86-29-82682132 (Tel), inquiry@medtl.com or contact your local distributor.

For up-to-date licensing information or product-specific disclaimers, please see the respective User Guide. Tianlong User Guides are available at www.medtl.net or can be requested from Tianlong Technical Services Support Center or the local distributor.

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