TIANLONG

# Avian Influenza Virus H5/H7/H9 Subtypes Nucleic Acid Detection Kit (Fluorescence PCR Method)

# User Guide

Σ 50 P941H

FOR ANIMAL USE ONLY!

Version 1.0

Qualitative In-Vitro Diagnostics / For use with qPCR Instruments compatible with
Avian Influenza Virus H5/H7/H9 Subtypes Nucleic Acid Detection Kit (Fluorescence PCR Method)

REF P941H

**Xi'an Tianlong Science and Technology Co., Ltd.**

No.4266, Shanglin Road, Weiyang District, Xi'an, 710021, Shaanxi, P.R. China

**IFU_P941H _EN / Oct. 2024**

TIANLONG

# Contents

## Introduction

Avian Influenza Virus (AIV) is a virus that mainly infects birds and belongs to the Orthomyxoviridae family. It can cause acute respiratory illness in birds and can lead to high mortality in severe cases. The virus has a variety of subtypes, the most common including H5, H7, H9 and other subtypes. AIV not only affect birds, but sometimes infect other animals, including humans. Human infection with avian influenza viruses usually results from direct contact with infected birds or their droppings. Although human cases of avian influenza are relatively rare, certain subtypes (e.g. H5N1, H7N9) can cause severe illness and high mortality when they infect humans. Therefore, it is necessary to combine the characteristics of the disease and use effective prevention and control measures to reduce the incidence and control the spread of the disease. Therefore, rapid and accurate diagnosis of AIV is very important. The ***Avian Influenza Virus H5/H7/H9 Subtypes Nucleic Acid Detection Kit*** developed by Tianlong Biotechnology assist in the diagnosis of Avian Influenza and public healthcare management.

## Intended Use

The Tianlong's ***Avian Influenza Virus H5/H7/H9 Subtypes Nucleic Acid Detection Kit*** is intended to be used for the qualitative detection of AIV H5, H7, H9 subtype RNA by Real-time reverse transcription Polymerase Chain Reaction (Real-time RT-PCR) method.

The test is designed to detect RNA from AIV in specimens include poultry serum, tissues, organs, excretory secretions, and swabs of cloaca and throat.

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

The Tianlong ***Avian Influenza Virus H5/H7/H9 Subtypes Nucleic Acid Detection Kit*** is to be used with Real-time PCR instruments with 4 or more fluorescence detection channels, which the test performance of the kit has been validated on. Such Real-time PCR thermal cyclers have appropriate fluorescence reading channels for FAM, VIC, Texas Red, Cy5 e.g. The reagent has been validated on Tianlong Gentier Real-time PCR system, Applied Biosystems™ QuantStudio™ 5 Real-Time PCR System and Applied Biosystems™ 7500 Real-Time PCR Systems.

## Kits Components

| **Ref no.** | | P941H |
|---|---|---|
| **Number of reactions** | | 50T |
| **RT-PCR reagents** | | |
| *AIV H5/H7/H9 Fluorescence Reaction Buffer* | 950 µL | 1 tube |
| *AIV H5/H7/H9 Enzyme Mixture* | 50 µL | 1 tube |
| **Controls** | | |
| *AIV H5/H7/H9 Positive Control* | 50 µL | 1 tube |
| *AIV H5/H7/H9 Negative Control* | 50 µL | 1 tube |
| *AIV H5/H7/H9 Internal Reference* | 250 µL | 1 tube |

**Note:** Store all reagents between -25°C to -15°C in a non-frost-free freezer. Do not mix the reagents from different batches. The negative control can be regarded as a "No Target Control" (NTC).

## Materials Required but Not Provided

- Microliter pipets* dedicated for PCR (0.1-2.5 µL; 1-10 or 1-20 µL; 20-200 µL; 1000 µL).
- Benchtop centrifuge* with rotor for 0.5 mL/1.5 mL reaction tubes (capable of attaining 10,000 rpm).
- Benchtop vortex mixer*.
- It is recommended to use a detection kit with Real-time PCR thermal cyclers with appropriate fluorescence reading channels for FAM, VIC, Texas Red, Cy5 dyes such as Applied Biosystems™ QuantStudio™ 5 Real-Time PCR System, Applied Biosystems™ 7500 Real-Time PCR Systems and Tianlong Gentier real-time PCR systems.

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

**Note:** please refer to relevant manufacturer's Instructions for Use (IFU) to proceed with the instrument.

## Principles of the Assay

The kit is designed with specific primers and specific probe on Avian Influenza Virus (AIV) H5, H7, H9 subtype conservative gene segment. The probe will have specific binding with one section of RNA template in middle of primer amplification area. In PCR extension reaction process, the excision enzyme activity of Taq enzyme will cut down 5'-end fluorophore from probe to make it free in reaction system and break away from shielding of 3'-end fluorescence quencher, which means it can accept the optical excitation, emit fluorescence for instrument test and achieve automatic test for AIV nucleic acid in totally enclosed reaction system by this way.

In this kit, a synthetic non-competitive sequence was designed as an internal reference template, which had no interference with the target gene. The specific primers and probes were designed according to this internal reference template. VIC channel was selected for internal control to achieve monitoring for test process in totally enclosed reaction system and effectively monitoring for occurrence of false negative.

## Reagent Storage and Handling

All reagents must be stored at -25°C to -15°C for 12 months. The stability of unspent reagents would not be influenced by re-storage. But the thawing and freezing should not be more than three times. The opened reagents should be placed no more than 8 hours at room temperature. The products should be shipped by ice box or refrigerated truck under 2°C to 8°C. Simulated transport tests indicate that the stability and validity could not be influenced by transport.

## Sample Requirements

The ***Avian Influenza Virus H5/H7/H9 Subtypes Nucleic Acid Detection Kit*** is designed to detect Avian Influenza Virus H5/H7/H9 Subtype in poultry tissues, organs, excretory secretions, and swabs of cloaca and throat.

Storage: samples can be stored at 2-8°C for no more than 24 hours; under -20°C for no more than 3 months; repeated freeze-thaw should be avoided.

Transportation: use a foam box with ice to seal for transportation.

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

## Before Starting

- Identify the product.
- Verify the expiration date.
- Verify the latest instruction for use available for the product lot number.
- Verify if the product was used already. If yes, check the remaining tests available.

## Nucleic Acid Extraction

Tianlong ***Avian Influenza Virus H5/H7/H9 Subtypes Nucleic Acid Detection Kit*** is compatible with RNA /nucleic acids of adequate quality prepared from intended samples using common RNA/nucleic acid extraction kits/methods. The prepared RNA/nucleic acids can be used directly as sample RNA/nucleic acid material, moved forward to the Real-time PCR reaction setup step.

**We recommend add 5μL AIV H5/H7/H9 Internal Reference to 200-400μL sample and extract together when extracting nucleic acid of samples (For Tianlong Animal Virus DNA and RNA Extraction Kit Series).**

1) Serum and vesicular or pustular lesion fluid samples: extract 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 it into pieces with surgical scissors and mix well; then, put 0.05-0.15 g into the grinder, add 1.5 mL normal saline and continue to grind it thoroughly; after homogenization, transfer it to a 1.5 mL sterilizing centrifuge tube, centrifuge it at 12,000 rpm for 5 min, take 400 μL supernate for nucleic acid extraction.

Positive Control and Negative Control do not need to be extracted and tested directly in each Real-time RT-PCR assay Run.

If under certain circumstances prepared RNA/nucleic acids need to be frozen stored for a later time testing, storage in a freezer of -70℃ or lower is recommended whenever possible for minimal nucleic acid degradation during storage. Repeated Freeze/Thaw of prepared sample RNA/nucleic acids should be avoided whenever possible.

## RT-PCR Reaction Setup

1. Thaw the following reagents on ice: ***AIV H5/H7/H9 Fluorescence Reaction Buffer*** and ***Enzyme Mixture***. Gently and evenly mix each individual reagent, then briefly centrifuge (2000rpm, 10sec) the reagents to collect the contents.
2. Set up a premix solution based on the number of sample preps to be tested. The volume of the premix required for all sample prep(s) to be tested = (number of sample preps + 2 controls) * the total volume of premix reagents (listed in Table 1).

| Premix reagents | Volume |
|---|---|
| *AIV H5/H7/H9 Fluorescence Reaction Buffer* | 19 µL |
| *AIV H5/H7/H9 Enzyme Mixture* | 1 µL |
| Total volume | 20 µL |

*Table 1 : Premix reagents*

3. Evenly aliquot 20 µL of the premix reagents into each PCR tube (one PCR tube per sample to be tested). Add 5 µL of each extracted RNA solution to a single qPCR tube. Do not add more than one sample of extracted RNA into a single qPCR tube. Add 5 µL in two distinct qPCR tubes of ***AIV H5/H7/H9 Positive Control*** and ***Negative Control*** (Positive Control and Negative Control do not require extraction), respectively. **Each qPCR tube shall have a total volume of 25 µL. Then immediately close the tubes and transfer the reaction setup into a qPCR machine for the amplification.**

## Thermal Cycler Settings

Set up the following thermal cycling program. It is recommended to use 4 or more fluorescence detection channels RT-PCR system.

| Stage | Number of Cycles | Temperature (℃) | 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 2 : RT-PCR Cycling program*

Selection of fluorescence channels： Selection of fluorescence channels：H9 subtype (FAM), H5 subtype (Texas Red/ROX), H7 subtype (Cy5) and Internal reference (VIC).

## Detection Channels

Four channels are used in this one-tube qPCR assay. It is recommended to perform the color (channel) calibration as requested by the instrument's manufacturer. Please refer to the instrument's user manual to perform this calibration. Choose the channels for each sample to be tested with Tianlong's ***Avian Influenza Virus H5/H7/H9 Subtypes Nucleic Acid Detection Kit.***

**Threshold Value Setting Principle:**

- Manual setting: set the threshold value a little bit greater than the max fluorescence value of the normal negative control amplification curve.
- Auto setting: the instrument automatically set the threshold value.

## Result Analysis

1. Negative control: there is no Ct value and amplification curve in any channel.
2. Positive control: there are typical S-shape amplification curves and Ct values are all≤ 30 in FAM, Texas Red/ROX, Cy5 and VIC channel.
3. The internal reference Ct value of the test samples should be ≤35. If there have no Ct value in the internal reference of 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 at the same time, or it is invalid.

**Result Interpretation:**

After the above quality control conditions are met, carry out the following analysis (in FAM channel is H9 subtype, in Texas Red/ROX channel is H5 subtype, in Cy5 channel is H7 subtype and in VIC channel is Internal Reference):

| FAM (H9) | Texas Red/ROX (H5) | Cy5 (H7) | VIC (Internal Reference) | Result |
|---|---|---|---|---|
| Ct <45 | Ct <45 | Ct <45 | Ct ≤35 | **H9 subtype and/or H5 subtype and/or H7 subtype Positive** |
| No Ct value | No Ct value | No Ct value | Ct ≤35 | **Negative** |
| Any result | Any result | Any result | No Ct value or Ct >35 | **Invalid test** need to be checked and retested. |

## Performance Characteristics

The following performance characteristics of the Tianlong's ***Avian Influenza Virus H5/H7/H9 Subtypes Nucleic Acid Detection Kit*** have been established following the procedure described in this datasheet.

**Non-clinical studies**

- Limit of detection: 500 copies/mL
- Specificity: There was no cross-reaction of other common pathogens with the same infection site or similar infection symptoms.
- Precision: The assay was used to respectively detect the precise reference specimens of high and low concentrations in different time ranges for 20 times, and the precision values of intra and inter Ct values were all <5%.

## Limitations

- All reagents in the kit are intended for in vitro diagnostic use as indicated.
- The test should be carried out by professionals adequately trained in professional lab 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 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 arise from natural mutations could potentially influence testing performance and result in false testing results. Up to today, results from bioinformatics analysis and comprehensive laboratory studies indicate that, partially due to the emphasis on mutation tolerance concept during assay design and development, this kit could tolerate currently known AIV mutations without obvious compromise on assay performance.
- Test results should be used in combination with clinical and epidemic information for medical decisions.

## Warnings and Precautions

**Laboratory precautions**

Use extreme caution to prevent:

- RNase contamination which might cause degradation of the template RNA
- RNA or PCR carryover contamination resulting in false positive signal

We therefore recommend the following:

- To make sure an accurate and reliable result, always use DNase/RNase-free disposable pipette tips, tubes and calibration pipettes.
- Use separated and segregated working areas: 1) Reagent preparation area – preparing the reagents for amplification, 2) sample preparation area- isolation of the RNA/ DNA from sample and control, and 3) Amplification area- amplification and detection of nucleic acid target.
- 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 glove, tubes, pipette and filter tips should not do contain Fluorescence material.
- Avoid the bubbles when separate the reaction solution into tubes. Check the tubes before amplification to avoid contamination induced by leak 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.
- Try to avoid the generation of air bubbles when the reaction solution is dispensed, and check whether the reaction tubes are tightly closed before loading on the machine to avoid the leakage contaminating the instrument.
- After the amplification, the reaction tube was taken out, sealed in a special plastic bag, and discarded at the designated place.
- The used tips should be thrown into disposal bottle which have 10% sodium hypochlorite solution and discarded with other 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 |

## Contact Information

For technical assistance and more information, please contact our Technical Support Center at +86-29- 82682132 (Tel), +86-29-82216680 (Fax), 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.