Duck-derived ingredients Nucleic Acid Detection Kit (Fluorescent PCR Method)

User Guide

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Version 1.0

Qualitative In-Vitro Diagnostics / For use with qPCR Instruments compatible with Duck-derived ingredients Nucleic Acid Detection Kit (Fluorescent PCR Method)

P597H

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Introduction

Animal-derived food has become an indispensable part of people's dietary structure because it is rich in essential nutrients for human beings. With the development of society and the substantial improvement of living standards, people's demand for animal-derived food is increasing. However, in recent years, the safety problems of animal-derived food caused by zoonotic diseases have become more and more serious, which has posed a certain threat to public health security. Therefore, it has attracted the attention of the whole society. The testing requirements for animal-derived ingredients such as animal feed are also increasing.

Since most meat products on the market have been minced, mixed, and processed at high temperature, it is difficult to identify the authenticity of the infiltrated ingredients through sensory inspection, even if they penetrate into meat with similar properties. Therefore, methods for identifying meat ingredients are urgently needed. With the development of biological technology, molecular biology identification methods based on genetic differences between species have become a hot spot in the identification of meat species.

The Duck-derived ingredients Nucleic Acid Detection Kit developed by Tianlong Biotechnology is designed to quickly and accurately detect Duck-derived ingredients in animal tissues, food, feed and other samples, and aims to meet the current needs of relevant food adulteration testing.

Intended use

The TianLong's Duck-derived ingredients Nucleic Acid Detection Kit is intended to be used for the qualitative detection of Duck-derived ingredients nucleic acid by fluorescence Polymerase Chain Reaction (PCR) method.

The test is designed to detect DNA from Duck-derived ingredients in animal tissues, food, feed and other samples. The test results are for veterinary clinical reference only and cannot be used as the basis for confirming or excluding cases alone.

The TianLong Duck-derived ingredients Nucleic Acid Detection Kit is to be used with Real-time PCR instruments with 2 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 (HEX) e.g. Applied Biosystems™ 7500 Real-Time PCR Systems, TianLong Gentier Real-time PCR systems, etc.

Kits components

Ref no.		P597H
Number of reactions		50T
RT-PCR reagents		
Duck Reaction Buffer	900 µL	1 tube
Duck Enzyme Mix	25 µL	1 tube
Duck Primer and Probe Mix	75 µL	1 tube
Controls		
Duck Positive Control	40 µL	1 tube
Negative Control	40 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 referred 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*
- qPCR instrument* with FAM, VIC (HEX) channels, i.e. Xi'an 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 primer and specific probe on Duck-derived ingredients mitochondrion gene segment. The probe will have specific binding with one section of DNA 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 Duck-derived ingredients gene nucleic acid in totally enclosed reaction system by this way.

This kit was designed with a synthetic, non-competitive sequence as an internal control that does not interfere with the target gene of Duck-derived ingredients. This sequence was entered into the NCBI website for BLAST comparison analysis, which confirmed that this sequence could not be found in the NCBI nucleic acid library. The primer and probe were designed based on this internal control, and the internal control was detected at HEX/VIC wavelength, thus enabling monitoring of the detection process in a fully closed reaction system, which can effectively monitor the occurrence of false negatives.

Sample requirements

1. Specimen: Food containing animal components, Animal tissue or blood, Animal feed, Meat products, etc.

2. Collection: for specific sampling method, please refer to the "Microbial Specimen Collection Manual".

3. Storage: samples can be stored at 2~8℃ for no more than 24 hours; under -20℃ for no more than 3 months; under -70℃ for long-time, but repeated freeze-thaw should be avoided.

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

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.

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.

Quantitative PCR (qPCR) Reaction Setup

1. Thaw the following reagents on ice: ***Duck Reaction Buffer***、***Duck Enzyme Mix*** and ***Duck Primer and Probe Mix*** . 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
Duck Reaction Buffer	18 μL
Duck Enzyme Mix	0.5 μL

Duck Primer and Probe Mix	1.5 µL
Total volume	**20 µL**

Table 1 : Premix reagents

3. Evenly aliquot the premix(es) into qPCR tube(s) (one qPCR tube per sample to be tested). Add 5 µL of each extracted DNA solution to a single qPCR tube. Do not add more than one sample of extracted DNA into a single qPCR tube. Add 5 µL in two distinct qPCR tubes of Duck 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.**

qPCR Cycling Condition

Set up the following thermal cycling program. It is recommended to use a 2-channels qPCR system.

Stage	Cycle	Temperature (°C)	Time (min:sec)
1	1	95	3:00
2	40	95	00:15
		60	00:30 (Fluorescence collection)

Table 2 : qPCR Cycling program

Selection of fluorescence channels： Duck-derived ingredients(FAM) and internal controls (VIC/HEX).

Detection Channels

Two 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 Duck-derived ingredients 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 typical S-shape amplification curve.
2. Positive control: there is typical S-shape amplification curve and Ct value is ≤32 in FAM channel, but there have no obvious exponential growth in VIC/HEX channel
3. The internal control Ct value of the test samples should be <40. If there have no Ct value in the internal control of the test sample , please find out the reasons and the retest the sample. (If use this kit to test non-Duck-derived samples, such as the environment, there is no Ct value for internal control.)
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 Duck-derived ingredients and in VIC/HEX channel is internal control):

FAM （Duck-derived ingredients）	VIC/HEX （Internal Control）	Result

Ct≤35	Ct＜40	**Duck-derived ingredients positive**
35<Ct≤40	Ct＜40	Retest. If the re-test result show that the Ct value is **still over than 35 to 40**, and the amplification curve is S shaped and show obvious exponential growth, then it is judged to be **positive to Duck-derived ingredients**; **otherwise** it is judged to be **negative to Duck-derived ingredients**.
No Ct value or Ct=40	Ct＜40	**Duck-derived ingredients Negative**
No Ct value or Ct=40	No Ct value or Ct=40	**Invalid test** and need to be checked and retested.

Performance Characteristics

The following performance characteristics of the TianLong's Duck-derived ingredients 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

Limits

● 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 IVD 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 Duck-derived ingredients 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 Duck-derived ingredients 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:

- **DNase contamination which might cause degradation of the template DNA**
- **DNA 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 fluorescent materials, disposable glove, tubes, pipette and filter tips should not do contain fluorescent material.
- Avoid the bubbles when separate the reaction solution into tubes. Check the tubes before amplification to avoid contamination induced by leak of fluorescent 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.

Contact Information

For technical assistance and more information, please contact our Technical Support Center at+86 0512-62525631 (phone), +86 0512-62956337 (fax), www.medtl.cnor contact your local distributor.

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