



Chicken Ingredients Nucleic Acid Detection Kit (Fluorescence PCR Method)

User Guide



For Research Use Only!

Version 2.1

For use with qPCR Instruments compatible with Chicken Ingredients Nucleic Acid Detection Kit (Fluorescence PCR Method)

REF

P596H

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Version	2.1
Change	Symbols

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 **Chicken Ingredients Nucleic Acid Detection Kit** developed by TianLong Biotechnology is designed to quickly and accurately detect Chicken 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 **Chicken Ingredients Nucleic Acid Detection Kit** is intended to be used for the qualitative detection of Chicken Ingredients DNA by Real-time Polymerase Chain Reaction (Real-time PCR) method.

The test is designed to detect DNA from Chicken ingredients in specimens such as food containing animal components, animal tissue or blood, animal feed, meat products, etc. collected from the samples to be tested or the samples that require Chicken origin identification.

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

The TianLong **Chicken 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, Cy5 e.g., Applied Biosystems™ 7500 Real-time PCR Systems, Tianlong Gentier Real-time PCR systems, etc.

Kits Components

Ref No.	P596H	
Number of Reactions	50T	
PCR Reagents		
REAG 1	750 µL	1 tube
REAG 2	500 µL	1 tube
Controls		
CONTROL +	40 µL	1 tube
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*.
- Extraction instrument*.
- qPCR instrument* with FAM,Cy5 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 Chicken Ingredients Nucleic Acid conservative 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 Chicken Ingredients 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 Chicken-derived component. 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 Cy5 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 °C for no more than 24 hours; under -20 °C for no more than 3 months; under -70 °C 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.

Nucleic Acid Extraction

TianLong **Chicken Ingredients Nucleic Acid Detection Kit** is compatible with DNA /nucleic acids of adequate quality prepared from intended samples using common DNA/nucleic acid extraction kits/methods. The prepared DNA/nucleic acids can be used directly as sample DNA/nucleic acid material, moved forward to the Real-time PCR reaction setup step. **We recommend adding 10 µL internal control to each 200 µL sample and extracting together when extracting nucleic acid from samples.**

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

If under certain circumstances prepared DNA/nucleic acids need to be frozen stored for a later time testing, storage in a freezer of -70°C or lower is recommended whenever possible for minimal nucleic acid degradation during storage.

Repeated Freeze/Thaw of prepared sample DNA/nucleic acids should be avoided whenever possible.

Quantitative PCR (qPCR) Reaction Setup

1. Thaw the following reagents on ice: REAG 1. Gently invert to mix the reagent, then briefly centrifuge (2000 rpm, 10 s) to let solutions be settled to the bottom of tubes before moving to the next step.
2. Prepare Master MIX based on the planned number of samples to be tested.

To calculate the volume of each reagent component required for Master MIX preparation, it needs to cover all the samples and controls to be tested in the assigned assay Run with reasonable extra set aside for operational tolerance.

In many cases, preparing Premix with 10-20% extra volume is a good practice.

3. 96-well PCR reaction plates or PCR reaction tube stripes could be used for reaction setup. Evenly aliquot 15 μ L of the prepared Master MIX into each PCR tube. Add 10 μ L of each extracted DNA nucleic acid solution to the designated PCR tube. Add 10 μ L of and to the respectively assigned tubes.

At the end of setup, each PCR tube shall have a total volume of 25 μ L.

Then immediately close/cover the tubes and transfer the reaction setup tube stripes/plate into a Real-time PCR cyclers for amplification reactions.

qPCR Cycling Condition

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

Table 1 : qPCR Cycling program

Stage	No. of cycles	Temperature*	Duration
1	1	50 °C	2 min
2	1	95 °C	20 s
3	42	95 °C	2 s
		60 °C	15 s** (fluorescence detection)

*:Tianlong Gentier Real-time PCR Systems are recommended for heating rate of 6 °C /s, and other instruments are selected according to the specific performance of the instrument.

** : Other instruments, such as ABI 7500, had a fluorescence setting of 31 s and had no effect on the results.

Assignment for Fluorescence Detection Channels:

- FAM channel for Target gene of Chicken ingredients
- Cy5 channel for Internal Control (IC)

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 **Chicken 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 ≤ 30 in FAM channel, but there is no obvious exponential growth in Cy5 channel.
3. The internal control Ct value of the test samples should be < 42 . If there is no Ct value in the internal control of the test sample, please find out the reasons and the 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 Chicken Ingredients and in Cy5 channel is internal control):

Table 2 The result of all channels Ct value interpretation

Channel	Positive (+)	Negative (-)
FAM (Chicken)	<ul style="list-style-type: none"> • Ct ≤ 38 • If the Ct value is > 38 and the retest result shows the Ct value is < 42 	<ul style="list-style-type: none"> • NO Ct Value or Ct=42 • If the Ct value is > 38 and the retest result shows the Ct value is > 42
Cy5(IC)	<ul style="list-style-type: none"> • Ct < 42 	<ul style="list-style-type: none"> • NO Ct Value or Ct=42

1. The result interpretation:

Table 3 The result interpretation of Chicken ingredients Nucleic Acid Detection Kit

Chicken (FAM)	IC (Cy5)	Result
+	+/-	Chicken ingredients POSITIVE
-	+	Chicken ingredients NEGATIVE
-	-	Invalid test and need to be checked and retested.

Performance Characteristics

The following performance characteristics of the TianLong's **Chicken 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 ingredients of animal origin.
- 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 $< 5\%$.

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 Chicken 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 Chicken 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,
 - 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.

Symbols

The following table describes the symbols that may appear on the labeling or in this document.

	Catalog number
	Batch code
	Contains reagents sufficient for <N> tests
	Use-by date
	Date of manufacture
	Caution
	Temperature limit
	Manufacturer
	Consult instructions for use
	Keep away from sunlight
	Fragile handle with care
	Content of the Kit
	P596H Master Mix (Chicken Master Mix)
	Internal Control
	P596H PC (Chicken Positive Control)
	P596H NC (Chicken Negative Control)
	Recycling symbol PAP21: non corrugated cardboard

References

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3. HERMAN, LIEVE (2001). Determination of the animal origin of raw food by species-specific PCR. *Journal of Dairy Research*, 68(3),-. doi:10.1017/S0022029901004940
4. Amaral, Joana S.; Santos, Cristina G.; Melo, Vitor S.; Costa, Joana; Oliveira, M. Beatriz P.P.; Mafra, Isabel (2015). Identification of duck, partridge, pheasant, quail, chicken and turkey meats by species-specific PCR assays to assess the authenticity of traditional game meat Alheira sausages. *Food Control*, 47, 190 – 195. doi:10.1016/j.foodcont.2014.07.009
5. User Protocol for Evaluation of Qualitative Test Performance; Approved Guideline-second edition. Clinical & Laboratory Standard Institute (CLSI): EP12-A2, 2008

6. Protocols for Determination of Limits of Detection and Limits of Quantitation; Approved Guideline; Clinical & Laboratory Standard Institute (CLSI): EP17A

Contact Information

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