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

User Guide

Version 1.0

IVD

Qualitative In-Vitro Diagnostics / For use with qPCR Instruments compatible with

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

REF P600H

Suzhou TianLong Biotechnology Co., Ltd.
NW-07-501&NE-33 Nanopolis, No.99 Jinjihu Road
Suzhou 215123 JiangSu Province, P.R. China

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.

The ***Cat-derived ingredients Nucleic Acid Detection Kit*** developed by TianLong Biotechnology is designed to quickly and accurately detect Cat-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 ***Cat-derived ingredients Nucleic Acid Detection Kit*** is intended to be used for the qualitative detection of Cat-derived ingredients nucleic acid by fluorescence Polymerase Chain Reaction (PCR) method.

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

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

Kits components

Ref no.		P600H
Number of reactions		50T
RT-PCR reagents		
P600H R S Master Mix	1000 µL	1 tube
Controls		
P600H P C	40 µL	1 tube
P600H N C	40 µL	1 tube
P600H I C	100 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, 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 Cat mitochondrion 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 Cat-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 the Cat genome. 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℃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.

Nucleic Acid Extraction

TianLong ***Cat-derived 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 RT-PCR reaction setup step.

We recommend add 2μL internal control to each 200μL sample and extract together when extracting nucleic acid of samples.

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 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.

Real-time RT-PCR Reaction Setup

1. Thaw the following reagents on ice: ***P600H R S Master Mix***. Gently and evenly mix the ***P600H R S Master Mix***, then briefly centrifuge (2000rpm, 10sec) the reagents to collect the contents.
2. Prepare ***P600H R S Master Mix*** based on the planned number of samples to be tested.To calculate the volume of each reagent component required for Premix 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, to prepare 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 20μL of the ***Master Mix*** into PCR tube(s). Add 5 μL of each extracted DNA solution to a single qPCR tube. Add 5 μL of **P600H P C** and **P600H N C** to the respectively assigned tubes. (Positive Control and Negative

4. Control do not require extraction).
   Each qPCR tube shall have a total volume of 25 µL.
   Then immediately close/cover the tubes and transfer the reaction setup tube stripes/plate into a Real-time PCR cycler for amplification reactions.
   Positive Control and Negative Control must be run in each assay Run.

Thermal Cycler Settings

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)

Selection of fluorescence channels： Cat-derived ingredients(FAM) and internal controls (Cy5).

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 ***Cat-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 Cy5 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 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 Cat-derived ingredients and in Cy5 channel is internal control):

FAM（Cat-derived ingredients）	Cy5（Internal Control）	Result
Ct≤35	Ct＜40	**Cat-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 Cat-derived ingredients**; **otherwise** it is judged to be **negative to Cat-derived ingredients**.
No Ct value or Ct=40	Ct＜40	**Cat-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 ***Cat-derived ingredients Nucleic Acid Detection Kit*** have been established following the procedure described in this datasheet.

- Limit of detection: 500 copies/mL.
- Specificity: There was no cross-reaction of other Animal-derived genes, such as Dog-derived component, Pig-derived component, Bovine-derived component, Sheep-derived component, Equine-derived component, Donkey-derived component, Chicken-derived component, Duck-derived component, Goose-derived component, Mouse-derived component, Deer-derived component, Fox-derived component. etc.
- 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%.

Quality Control

In accordance with the ISO 13485:2016 Medical devices— Quality management systems and TianLong ***Cat-derived ingredients Nucleic Acid Detection Kit*** Quality Control Program, each batch of the ***Cat-derived ingredients Nucleic Acid Detection Kit*** is tested against predetermined specifications to ensure consistent product quality.

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.

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.

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
	Caution
	Temperature limit
	In vitro diagnostic medical device
	Manufacturer
	Do not re-use

Contact Information

For technical assistance and more information, please contact our Technical Support Center at

+86-029-82682132(Tel) , inquiry@medtl.com(Mail), www.tlgenetech.cn(Website).

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