Horse Ingredients Nucleic Acid Detection Kit (Fluorescent PCR Method)

User Guide

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For Research Use Only !

Version 1.0

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

REF P594H

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Introduction

At present, domestic, and foreign meat is adulterated and shoddy incidents are emerging one after another, which has seriously damaged the confidence of consumers and the rights and interests of legitimate businesses. In 2013, European frozen food processors and suppliers announced that horse meat was detected in several frozen beef products in France, the United Kingdom and Sweden. In 2017, 10% of beef samples collected in the Americas and Mexico also contained horse meat. Since horses may take medicines that are not conducive to food safety, such as clenbuterol, it is difficult to guarantee food safety. Consumers generally worry that the analgesic ingredients that may be contained in horse meat are harmful to the human body. Therefore, it is of great social significance to strengthen the detection of horse ingredients.

Since most meat products on the market in my country 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 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.

To understand the impact of the European horse meat storm incident on China's imports of livestock meat products from the European Union, grasp the adulteration of horse meat in domestic beef and mutton.

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

The test is designed to detect DNA from Horse 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 ***Horse Ingredients Nucleic Acid Detection Kit i***s 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.		P594H
Number of reactions		50T
PCR reagents		
Horse Reaction Buffer	725 µL	1 tube
Horse Enzyme Mix	25 µL	1 tube
Controls		
Horse Positive Control	40 µL	1 tube
Negative Control	40 µL	1 tube
Internal Control	500 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 to 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, 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 Horse ingredients mitochondrion gene segment. The probe will have specific binding with one section of DNA template in the 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 Horse 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 Horse 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.

Nucleic Acid Extraction

TianLong ***Horse 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 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.

Qualitative PCR (qPCR) Reaction Setup

1. Thaw the following reagents on ice: ***Horse Reaction Buffer and Horse Enzyme Mix***. Gently and evenly mix each individual reagent, then briefly centrifuge (2000 rpm, 10 sec) 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
Horse Reaction Buffer	14.5 µL
Horse Enzyme Mix	0.5 µL
Total volume	**15 µL**

Table 1 : Premix reagents

3. Evenly aliquot the premix(es) into qPCR tube(s) (one qPCR tube per sample to be tested). Add 10 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 10 µL in two distinct qPCR tubes of Horse 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.

Table 2: qPCR Cycling program

Stage	No. of cycles	Temperature	Rate*	Duration
1	1	50 ℃	6℃/S	2 min
2	1	95 ℃	6℃/S	20 s
3	42	95 ℃	6℃/S	2 s
		60 ℃	6℃/S	15 s** (fluorescence detection)

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

**: Other instruments, such as ABI7500, had a fluorescence setting of 31s and had no effect on the results.

Assignment for Fluorescence Detection Channels:

- FAM channel for Target gene of Horse ingredients
- VIC/HEX 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 ***Horse 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 has no obvious exponential growth in VIC/HEX channel.
3. The internal control Ct value of the test samples should be <42. 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-horse 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 Horse ingredients and in VIC/HEX channel is internal control):

Table 3 The result of all channels Ct value interpretation

Channel	Positive (+)	Negative (-)
FAM (horse)	• Ct ≤ 38 • If the Ct value is >38 and the retest result show the Ct value is <42	• NO Ct Value or Ct=42 • If the Ct value is >38 and the retest result show the Ct value is >42
VIC/HEX（IC）	• Ct < 42	• NO Ct Value or Ct=42

1. The result interpretation:

Table 4 The result interpretation of Horse Ingredients Nucleic Acid Detection Kit

Horse (FAM)	IC(VIC/HEX)	Result
+	+/-	Horse ingredients POSITIVE
-	+	Horse ingredients NEGATIVE
-	-	**Invalid test and need to be checked and retested.**

Performance Characteristics

The following performance characteristics of the TianLong's ***Horse 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 Horse 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 Horse ingredient 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.

Symbols

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

REF	Catalog number
LOT	Batch code
Σ <N>	Contains reagents sufficient for <N> tests
	Use-by date
	Caution
	Temperature limit
	Manufacturer
	Consult instructions for use
	Keep away from sunlight
	Fragile handle with care
21 PAP	Recycling symbol PAP21: non corrugated cardboard

References

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Contact Information

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