



Cervine Ingredients Nucleic Acid Detection Kit (Fluorescence PCR Method)

User Guide



For Research Use Only!

Version 2.0

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

REF

P602H



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| Version | 2.0 |
| Change | Address of Manufacturer Chapter "Kits components" IC Channel "VIC/HEX" → "Cy5" Chapter "Symbols" Chapter "Contact Information" |

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 detection of Cervine Ingredients refers to the application of the detection of whether there is Cervine nucleic acid in the tested samples. It is mainly used in the detection of animal tissue Ingredients, food testing, meat product testing, feed, etc. Important safety inspection items such as customs inspection and quarantine.

This kit is suitable for the detection of Cervine DNA nucleic acid in suspected samples and is used for the auxiliary detection of Cervine Ingredients. The test results are for reference only.

Intended Use

The TianLong **Cervine Ingredients Nucleic Acid Detection Kit** is intended to be used for the qualitative detection of Cervine Ingredients DNA by Real-time Polymerase Chain Reaction (Real-time PCR) method.

The test is designed to detect DNA from Cervine 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 Cervine 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 **Cervine 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. | | P602H |
|----------------------------|--------|--------|
| Number of Reactions | | 50 T |
| 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 10,000rpm).
- 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 Cervine 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 Cervine 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 Cervine-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 ***Cervine 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 freezing/thawing 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 set up, 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 cycler 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 ABI7500, 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 Cervine 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 **Cervine Ingredients Nucleic Acid** **Cervine Ingredients Nucleic Acid Detection Kit - User Guide**

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 sets 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 has 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 Cervine Ingredients and in Cy5 channel is internal control):

Table 2 The result of all channels Ct value interpretation

| Channel | Positive (+) | Negative (-) |
|------------------|--|--|
| FAM (Cervine) | <ul style="list-style-type: none"> • $Ct \leq 38$ • If the Ct value is $38 < Ct < 42$ 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 $38 < Ct < 42$ 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 Cervine ingredients Nucleic Acid Detection Kit

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

Performance Characteristics

The following performance characteristics of the TianLong **Cervine 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 $\leq 5\%$.

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 Cervine 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 Cervine 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 |
|  | P602H Master Mix (Cervine Master Mix) |
|  | Internal Control |
|  | P602H NC (Cervine Negative control) |
|  | P602H PC (Cervine Positive control) |
|  | Recycling symbol PAP21: non corrugated cardboard |

References

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

Contact Information

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

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