ICS 7.08
CCS A40

NATIONAL STANDARD OF THE PEOPLE'S REPUBLIC OF CHINA

GB/T 38164-2019

Identification of animal ingredients from common livestock and poultry - Real-time PCR

常见畜禽动物源性成分检测方法 实时荧光PCR法

Issued on: October 18, 2019 **Implemented on: October 18, 2019**

Issued by: State Administration for Market Regulation;
Standardization Administration of the PRC

Table of Contents

1 Scope

China's national method for identifying animal ingredients from common livestock and poultry species by real-time PCR. It specifies the terms and definitions, the abbreviations, the principle, the primers and probes, and the procedure. The method answers a question that food inspection asks constantly: what species is this meat. DNA survives processing that destroys every other identifying characteristic - mincing, cooking, emulsifying into a sausage - and species-specific PCR detects it in a few hours. The applications are commercial fraud, where a cheaper species is substituted for a dearer one; religious and dietary compliance, where the presence of pork or its absence is the whole question; and allergen and feed control. The horsemeat episode in Europe made the technique routine everywhere, and the reason it is written as a standard rather than left to laboratories is that the primer sequences and the reaction conditions determine whether a result can be relied on.

This Standard specifies the real-time PCR for the identification of animal ingredient from common livestock and poultry. This Standard applies to the Taqman probe real-time PCR

qualitative determination of species ingredients from cattle, yak, buffalo, sheep, goat, pig, camel, red deer, sika deer, reindeer, rabbit, dog, chicken, duck, goose, quail, pigeon, turkey, cat, fox, mink, raccoon, and rat in meat and processed products, offal, milk, and animal feed. Limit of detection (LOD) 1% (mass fraction).

2 Normative references

The following documents, in whole or in part, are normatively referenced in this document and are indispensable for its application. For dated references, only the edition cited applies. For undated references, the latest edition of the referenced document (including any amendments) applies.

GB/T 6682, Water for analytical laboratory use - Specification and test methods

GB/T 27403-2008, Criterion on quality control of laboratories - Molecular biological testing of food

GB/T 34796, Quantification and purity analysis of nucleic acid concentration in solution - Ultraviolet spectrophotometry

GB/T 35918, Identification of animal origin in animal products by DNA barcoding - Sanger sequencing

3 Terms and definitions, abbreviated terms

3.1 Terms and definitions For the purposes of this document, the following terms and definitions apply.

3.1.1 real-time PCR Add a fluorescent group to the PCR reaction system, and monitor the entire PCR amplification process in real time by the accumulation of fluorescence signals.

3.1.2 cycle threshold The number of cycles required for the fluorescence signal in each reaction tube to reach the set threshold.

3.2 Abbreviated terms For the purposes of this document, the following abbreviated terms apply. ATP8.ATP synthase subunit 8 COI. Cytochrome Coxidase I CTAB. Cetyltrithylammonium bromide cytb. Cytochrome b DNA. Deoxyribonucleic acid D-loop. Displacement loop region EDTA. Ethylene diaminetetraacetic acid Na2-EDTA. Ethylenediaminetetraacetic acid disodium salt ND

4 Principle

Perform real-time PCR amplification of animal ingredient from livestock and poultry using species-specific primers and probes; use the fluorescence signal of each cycle product in the PCR amplification reaction for identification, enabling qualitative detection of animal ingredient from common livestock and poultry.

5 Primers and probes for detection

The internal reference primer and probe sequences and common livestock and poultry-specific primer and probe sequences are shown in Table 1.

6.3 Absolute ethanol.

6.4 Sodium hydroxide solution (10 mol/L). Add

80.0 g of sodium hydroxide (NaOH) to 160 mL of double-distilled water; dissolve; cool to room temperature; then add double-distilled water to fix he volume to 200 mL.

6.5 Na2-EDTA solution (500 mmol/L, pH 8.0). Weigh

18.6 g of Na2-EDTA; add it to 70 mL of double-distilled water; then add an appropriate amount of sodium hydroxide solution (6.4); heat until it is completely dissolved; cool to room temperature; use sodium hydroxide solution (6.4) to adjust the pH to 8.0; add double-distilled water to fix the volume to 100 mL. Sterilize at 103.4 kPa (121 °C) for 20 min.

6.6 Tris-HCl solution (1 mol/L, pH 8.0). Weigh 121.1 g of Tris and dissolve it in 800 mL of double-distilled water; use HCl to adjust the pH to 8.0; add double-distilled water to bring the volume to 1000 mL. Sterilize at 103.4 kPa (121 °C) for 20 min.

6.7 CTAB extraction buffer (pH 8.0). Weigh

4.0 g of CTAB and

16.364 g of NaCl; add 20 mL of 1 mol/L Tris-HCl solution (pH 8.0) (6.6) and 8 mL of 500 mmol/L Na2-EDTA solution (pH 8.0); dissolve in 70 mL of double-distilled water; then bring the volume to 200 mL and sterilize at 103.4 kPa (121 °C) for 20 min.

6.8 CTAB precipitate. Weigh

1.0 g of CTAB and

0.467 g of NaCl; dissolve them in 70 mL of double-distilled water; then bring the volume to 200 mL; sterilize at 103.4 kPa (121°C) for 20 min.

6.9 Proteinase K (670 U/mL). Weigh

0.10 g of proteinase K dry powder with an enzyme activity of

33.5 U/mg (Unit, enzyme unit); add 5 mL of double-distilled water; shake gently until proteinase K is completely dissolved; aliquot into small portions and store at -20 °C.

6.10 PCR Master Mix (2x), or an equivalent real-time PCR premix can be used.

7 Instruments and equipment

7.1 Real-time PCR instrument.

7.2 Nucleic acid protein analyzer or ultraviolet spectrometer.

7.3 Constant temperature water bath.

7.4 Centrifuge. centrifugal force >=12 000 g.

7.5 pH meter. grade 0.01.

7.6 Balance. sensitivity

0.1 g,

0.01 g,

0.001 g.

8.1 Sample pretreatment

8.1.1 General Pre-treat the samples; then divide them into three equal parts, including the sample to be tested, the sample to be retested, and the sample to be retained.

8.1.2 Solid samples Use 70% ethanol and double-distilled water to rinse 2 ~ 3 times respectively; then collect in a clean 50 mL centrifuge tube or a clean sealed bag; freeze at -20 °C or below.

8.1.3 Semi-solid and liquid samples After mixing, dispense directly into clean 50 mL centrifuge tubes or clean sealed bags; store at -20 °C or below.

8.2 DNA extraction Extract according to the method of GB/T 35918. Alternatively, exact DNA using an equivalent DNA extraction kit.

8.3 Determination of DNA concentration and purity Determine and calculate the concentration of DNA according to the method in GB/T 34796, and determine the purity of the DNA.

Note. Before performing a fluorescence PCR amplification test, the DNA concentration should be diluted to the range of 5 ng/μL ~ 50 ng/μL.