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**INDUSTRY STANDARD OF THE
PEOPLE'S REPUBLIC OF CHINA**

SN/T 5226-2019

**Detection of turtle-derived components in exported foods
Real-time fluorescence PCR method
(English Translation)**

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Foreword

This standard was drafted in accordance with the rules given in GB/T 1.1-2009.

1 Scope

This standard specifies the real-time fluorescent PCR detection method for turtle-derived components in exported food.

This standard is only applicable to Chinese soft-shelled turtle, yellow-sand soft-shelled turtle, black soft-shelled turtle, pearl soft-shelled turtle, mountain turtle, spiny soft-shelled turtle, small soft-shelled turtle, Indian small-headed soft-shelled turtle and Qualitative detection of the components of the Burmese marginal soft-shelled turtle and other soft-shelled turtles.

2 Normative references

The following documents are indispensable for the application of this document. For dated reference documents, only the dated version applies to this article. For undated reference documents, the latest version (including all amendments) is applicable to this document.

GB/T 6682 Analytical laboratory water specifications and test methods GB 19489 Laboratory Biosafety General Requirements GB/T 27403-2008 Laboratory Quality Control Specification Food Molecular Biology Testing

3 Terms and definitions

The following terms and definitions apply to this document.

Trionyx Sinensis

It is a class of amphibians and reptiles in the subfamily Trionyx subfamily of the Reptilidae Turtles. Its meat is rich in nutrients and is a good nourishing product.

Turtles are also used as medicinal materials.

Polymerase chain reaction; PCR the template gene sequence is first denatured by high temperature to become single-stranded. Under the action of DNA polymerase and suitable reaction conditions, according to the template sequence the two designed primers anneal to the corresponding complementary sequences on the two strands of the template DNA, and then bind to each other.

Under the action of synthase, four kinds of deoxyribonucleic acid (dNTP) are used as substrates to extend the primers, and then repeat denaturation, annealing and Extend this cycle, so that the gene fragments to be amplified will be amplified geometrically.

Real-time fluorescent PCR Fluorescent groups are added to the PCR reaction system, and the accumulation of fluorescent signals is used to monitor the entire PCR process in real time.

Ct value cycle threshold the number of cycles experienced when the fluorescence signal in each reaction tube reaches the set threshold.

4 Abbreviations

The following abbreviations apply to this document.

DNA. deoxyribonucleic acid, deoxyribonucleic acid.

PCR. polymerase chain reaction, polymerase chain reaction.

dNTP. deoxyribonucleoside triphosphate, deoxyribonucleoside triphosphate.

dATP. deoxyadenosine triphosphate, deoxyadenosine triphosphate.

dCTP. deoxycytidine triphosphate, deoxycytidine triphosphate.

dGTP. deoxyguanosine triphosphate, deoxyguanosine triphosphate.

dTTP. deoxythymidine triphosphate, deoxythymidine triphosphate.

Tris. Tris (Hydroxymethyl) aminomethane.

EDTA. Ethylene diaminetetraacetic acid, ethylene diamine tetraacetic acid.

Taq. *Thermus aquaticus*, *Thermus aquaticus*.

OD. optical density, optical density.

FAM. Carboxyfluorescein,.

BHQ1.Black hole quencher 1, black hole quencher group 1.

5 Method summary

After the sample is ground, DNA is extracted, the DNA is used as a template, and the specific detection primers for the mitochondrial cytochrome b gene of the soft-shelled turtle and Probe, perform real-time fluorescent PCR amplification, observe the amplification phenomenon of real-time fluorescent PCR, and judge whether there are turtle components in the sample.

6 Reagents and materials

Unless otherwise specified, all reagents are analytical reagents or biochemical reagents. The experimental water meets the requirements of GB/T 6682.All reagents are Dispense with DNase-free containers.

6.1 Primers and probes for detection

The primers and probes for the amplification of soft-shelled turtle components are shown in Table 1.See Appendix A for the target sequence amplified by the turtle primer.

6.2 Reagents

6.2.1

Tissue lysate. 1 mol/L Tris-HCl (pH 8.0); 0.5 mol/L EDTA (pH 8.0); 10% (mass concentration) SDS.

6.2.2

Proteinase K solution (20 mg/mL).

6.2.3

Saturated phenol.

6.2.4

Trichloromethane.

6.2.5

Isoamyl alcohol.

6.2.6 75% ethanol.

6.2.7

NaAc solution (3 mol/L).

6.2.8

TE buffer (Tris-HCl, EDTA buffer). 10 mmol/L Tris-HCl (pH 8.0), 1 mmol/L EDTA (pH 8.0).

6.2.9

Real-time fluorescent PCR reaction mixture. 12.5 µL reaction system includes 1U~3 U Taq enzyme, 1xPCR buffer, 2.5 mmol/L~ 4.0 mmol/L Mg²⁺, 0.2 mmol/L dNTP. If there are equivalent commercial kits, these equivalent products can also be used.

7 Apparatus and equipment

7.1

Real-time fluorescent PCR machine.

7.2

Centrifuge. the centrifugal speed is not less than 12 000 r/min.

7.3

Constant temperature water bath.

7.4

Balance. Sensitivity 0.1 g, 0.01 g.

7.5

Micropipette. 10 µL, 100 µL, 200 µL, 1000 µL.

7.6

Nucleic acid protein analyzer or ultraviolet spectrophotometer.

7.7

Vortex oscillator.