

ICS 65.020.30
CCS B 41

SN

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

SN/T 5202-2020

**Technical Specification for Sika Deer Species Identification -
Real-time Fluorescent PCR Method**

梅花鹿物种鉴定技术规范-实时荧光PCR法
(English Translation)

Issued on: December 30, 2020

Implemented on: July 1, 2021

Issued by: General Administration of Customs

Table of Contents

Foreword

1 Scope

2 Normative references

3 Terms and definitions

4 Abbreviations

5 Reagents and materials

5.1 Primers and probes for detection

5.2 Reagents

6 Equipment

6.1 Real-time fluorescent PCR instrument

6.2 Centrifuge (centrifugal force 12 000 g)

6.3 Centrifuge tube (1.5 mL, 2.0 mL)

6.4 Nucleic acid protein analyzer or UV spectrophotometer

6.5 Micropipette (2.5 μ L, 20 μ L, 200 μ L, 1 000 μ L)

6.7 Constant temperature water bath (10 $^{\circ}$ C~90 $^{\circ}$ C)

6.8 Balance (sensitivity 0.01 g)

7 Inspection steps

7.1 DNA extraction of samples

7.2 Real-time fluorescent PCR detection

8 Judgment and expression of results

Foreword

This document is drafted in accordance with GB/T 1.1-2020.

This document was proposed and managed by the General Administration of Customs of the People's Republic of China.

Drafting organization of this document. Changchun Customs of the People's Republic of China.

1 Scope

This document specifies the real-time fluorescent PCR method for sika deer species identification.

This document applies to sika deer-derived products (sika deer antler, sika deer blood, sika deer placenta, sika deer support and other non-deep processed products) Qualitative testing.

2 Normative references

The contents of the following documents constitute the indispensable clauses of this document through normative references in the text. Among them, dated quotations Only the version corresponding to that date is applicable to this document; for undated references, the latest version (including all amendments) is applicable Used in this document.

GB/T 6682 Analytical laboratory water specifications and test methods

3 Terms and definitions

The following terms and definitions apply to this document.

Ct value clycle 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 documents.

PCR. polymerase chain reaction.

DNA. Deoxyribonucleic acid (deoxyribonucleic acid).

dNTP. deoxy-ribonucleoside triphosphate.

CTAB. cetyltrimethylammonium ammonium bromide.

Tris. Tris-(hydroxymethyl)-aminometha.

EDTA. ethylenediaminetetraacetic acid (ethylenediaminetetraacetic acid).

Taq. Thermus aquaticus (thermusaquaticus).

UNG. uracil-N-glycosylase (uracil-N-glycosylase).

OD. optical density (optical density).

ddH₂O. double distilled water.

5 Reagents and materials

Unless otherwise specified, the reagents are analytical reagents or biochemical reagents, and the experimental water meets the requirements of GB 6682.

5.1 Primers and probes for detection

Refer to Appendix A for the reference sequence of sika deer gene expansion target.

5.2 Reagents

5.2.1

CTAB extraction buffer. 20 ng/L CTAB, 0.1mol/L Tris-HCl, 1.4 mol/L NaCl, 0.02 mol/L EDTA, pH 8.0

5.2.5 Isopropanol

5.2.6 70% ethanol

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

5.2.8 Fluorescence quantitative PCR master mix. 10 μ L The reaction system includes. 1 U~2 U Taq enzyme, 1xPCR buffer, 2.5 mmol/L~4.0 mmol/L Mg^{2+} , 0.2 U~1 U UNG enzyme, 0.2 mmol/L d(A, C, G)TPs, 0.2 mmol/L~0.4 mmol/L dUTP, 400 nmol/L ROX dye (some fluorescent PCR machines do not require ROX calibration). If there is an equivalent commercial kit, then these equivalent products can be used.

6.5 Micropipette (2.5 μ L, 20 μ L, 200 μ L, 1 000 μ L)

6.6 Mortar or crushing device.

7.1 DNA extraction of samples

Weigh 200 mg or 200 μ L of the ground sample into a 2.0 mL centrifuge tube, and add 1.5 mL CTAB extraction buffer and 10 μ L of proteinase K solution, 65°C for 30 min, shake and mix from time to time; centrifuge at 13,000 g for 10 min, transfer the supernatant to a clean centrifuge tube, add 750 μ L of chloroform/isoamyl alcohol (24:1, V/V), mix well, centrifuge at 13,000 g for 5 min, and transfer the supernatant to a clean centrifuge tube; add 2 times the volume of CTAB precipitation solution, stand still at room temperature for 60 minutes, centrifuge at 13,000 g for 15 minutes, and discard the supernatant. Join 350 μ L of NaCl solution will resuspend the pellet. Then add 350 μ L of chloroform, vortex to mix, and centrifuge at 13,000 g for 10 min.

After transferring the supernatant, add 0.6 times the volume of isopropanol to precipitate the nucleic acid, leave it at room temperature for 20 minutes, centrifuge at 13,000 g for 10 minutes, and discard the supernatant.

Add 500 μ L of 70% ethanol to wash once and dry; add 100 μ L of TE to dissolve the precipitate. Use immediately or store at -20°C for later use.

An equivalent DNA extraction method can also be used to extract the DNA template.

7.2 Real-time fluorescent PCR detection

7.2.1

Amplification reaction system.

The volume is 20 μL . 2x fluorescence quantitative PCR master mix 10 μL , primer pair (10 nmol/L) each 1.0 μL , template DNA (100 ± 50 ng DNA) 2.5 μL , fluorescent probe (10 nmol/L) 0.5 μL , ddH₂O 5.0 μL .

7.2.2

Reaction conditions.

50 °C, 2 min; 95 °C, 10 min; 95 °C, 15 s, 60 °C, 1 min, 40 cycles. The reaction cycle parameters can be based on Make appropriate adjustments due to the different models of the thermal cycler.

7.2.2 Setting of control in the reaction system

In the detection process, a positive control, a negative control and a blank control were set up respectively. Use samples known to contain sika deer or sika deer-derived ingredients as Positive control, use a sample known to not contain sika deer-derived components as a negative control, and use an equal volume of ddH₂O instead of template DNA as a blank Contrast.

8 Judgment and expression of results

Real-time fluorescent PCR qualitative test

8.1

If the Ct value is ≤ 35 and the negative control, positive control and blank control results are normal, it can be determined that the sika deer source is detected in the sample Sexual component.

8.2

If the Ct value is ≥ 40 and the negative control, positive control and blank control results are normal, it can be determined that no sika deer is detected in the sample Source ingredients.

If the control result is normal, it can be determined that sika deer-derived components are detected in the sample. The Ct value of the re-amplification >40 , and the negative control, photo and empty If the white control result is normal, it can be determined that no sika deer-derived components are detected in the sample.

Appendix A