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

SN/T 5227.8-2019

**Rapid detection of donkey-derived components in exported
foods Recombinase-mediated strand replacement nucleic acid
amplification method (RAA method)**

(English Translation)

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Table of Contents

Foreword	
1 Scope	
2 Normative references	
3 Terms, definitions and abbreviations	
3.1 Terms and definitions	
3.2 Abbreviations	
4 Method summary	
5 Reagents and materials	
6 Equipment	
7 Detection steps	
7.1 DNA extraction	
7.2 Determination of DNA concentration and purity	
7.3 Real-time fluorescent RAA amplification	
8 Quality control	
8.1 Real-time fluorescent PCR instrument	
8.2 Constant temperature fluorescence detector	
9 Judgment and expression of results	
9.1 Judgment of results	
9.2 Presentation of results	
10 Measures to prevent cross contamination during testing	

Foreword

SN/T 5227-2019 "Rapid Detection of Animal-derived Components in Exported Foods Recombinase Mediated Strand Replacement Nucleic Acid Amplification Method (RAA Law)" is expected to be divided into the following parts.

--Part 1: Rapid detection of chicken-derived components in exported foods, recombinase-mediated strand replacement nucleic acid amplification method (RAA method);

--Part 2: Rapid detection of pig-derived components in export foods, recombinase-mediated strand replacement nucleic acid amplification method (RAA method);

--Part 3: Rapid detection of sheep-derived components in export foods

recombinase-mediated strand replacement nucleic acid amplification method (RAA method);

--Part 4: Rapid detection of duck-derived components in export foods, recombinase-mediated strand replacement nucleic acid amplification method (RAA method);

--Part 5: Rapid detection of bovine-derived components in exported foods, recombinase-mediated strand replacement nucleic acid amplification method (RAA method);

--Part 6: Rapid detection of buffalo-derived components in exported foods, recombinase-mediated strand replacement nucleic acid amplification method (RAA method);

--Part 7: Rapid detection of horse-derived components in export foods, recombinase-mediated strand replacement nucleic acid amplification method (RAA method);

--Part 8: Rapid detection of donkey-derived components in export foods, recombinase-mediated strand replacement nucleic acid amplification method (RAA method);

--Part 9: Rapid detection of fox-derived components in export foods, recombinase-mediated strand replacement nucleic acid amplification method (RAA method);

--Part 10: Rapid detection of mink-derived components in exported foods, recombinase-mediated strand replacement nucleic acid amplification method (RAA method);

--Part 11: Rapid detection of rat-derived components in exported foods
Recombinase-mediated strand replacement nucleic acid amplification method (RAA method).

This part is part 8 of SN/T 5227-2019.

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

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

Drafting organizations of this section. Zhengzhou Customs of the People's Republic of China, Chinese Academy of Inspection and Quarantine, China Animal Health and Epidemiology Center for Disease Research, Zhejiang Institute of Food and Drug Inspection, Dalian Customs of the People's Republic of China, Xiamen Customs of the People's Republic of China, China Beijing Customs of the People's Republic of China, Gongbei Customs of the People's Republic of China, Hangzhou Customs of the People's Republic of China, Fuzhou Sea of the People's Republic of China Off, Hangzhou Zhongce Biological

Technology Co., Ltd.

1 Scope

This part of SN/T 5227-2019 specifies the RAA detection method for donkey-derived ingredients in exported food.

This section applies to the qualitative detection of donkey-derived ingredients in exported food.

2 Normative references

The minimum detection limit (LOD) of the method specified in this section is 0.1% (W/W).

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/T 27403-2008 Laboratory Quality Control Specification Food Molecular Biology Testing

3.1 Terms and definitions

The following terms and definitions apply to this document.

3.1.1

Donkey *Equus asinus*

Perissodactyla, Equatiformes, Equidae, Equidae, Equus, and horses belong to the same genus *Equus*.

3.1.2

Real-time fluorescence RAA real-time RAA A constant-temperature nucleic acid rapid amplification technology (referred to as RAA technology) that recombinase-mediated strand replacement. Utilize what is obtained from bacteria or fungi Recombinant enzyme, at a constant temperature (usually 37 °C ~ 42 °C), the recombinase can be tightly combined with the primer to form a polymer of the enzyme and the primer.

With the help of the single-stranded DNA binding protein, the double-stranded structure of the template DNA is opened. When the primer is searched on the template DNA, it is completely matched with it.

When matching complementary sequences, under the action of DNA polymerase, new complementary DNA strands are formed, and the amplified products grow exponentially. Use fluorescent with the labeling of the optical probe, as the RAA reaction proceeds, the

RAA product and the increase in the fluorescence signal show a corresponding relationship.

3.2 Abbreviations

The following abbreviations apply to this document.

4 Method summary

Using the extracted DNA as a template, using donkey-specific detection primers and probes for real-time fluorescence RAA amplification, according to the real-time fluorescence the increase in RAA enables the detection and identification of donkey components in food and feed.

5 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.

Note: See Appendix A for the target gene sequence. F is the upstream primer, R is the downstream primer, P is the probe, FAM-dT, THF, BQH-dT and C3spacer all are probe modification groups.

5.1

CTAB extraction buffer (pH8.0). 10 g/L CTAB, 0.7 mol/L NaCl, 0.05 mol/L Tris-HCl, 0.01 mol/L Na₂EDTA.

5.2

Phenol. Chloroform. Isoamyl alcohol=25.24.1.

5.3

Isopropanol.

5.4 70% ethanol (volume ratio).

5.5

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

5.6

Buffer A. 20% polyethylene glycol.

5.7

Real-time fluorescent RAA amplification system. 2.5 mmol/L dNTPs, 225 ng/μL SSB, 300 ng/μL recA recombinase protein (SC-recA/BS-recA), 75 ng/μL Bsu DNA polymerase, 75 ng/μL Exo exonuclease, 250 mmol/L Tricine, 12.5 mmol/L dithiothreitol, 250 ng/μL creatine