

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

SN/T 5145.1-2019

**Rapid Identification of animal ingredient in export food and feed
-- Part 1: Detection of Felinae Ingredient PCR-Lateral flow
dipstick 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 principle
5 Reagents and materials
5.2 Reagents
6 Equipment
6.7 Nucleic acid protein analyzer or UV spectrophotometer
7 Inspection steps
7.1 Sample preparation
7.2 DNA extraction
7.3 Determination of DNA concentration and purity
7.4 PCR amplification
8 Quality control
9 Results judgment and report
9.1 Determination of results
9.2 Results report
10 Measures to prevent cross-contamination during testing
11 Waste disposal
12 Detection limit

Foreword

Sn/t 5145 "Method for rapid detection of animal-derived ingredients in exported food and feed" is divided into the following 13 parts.

- Part 1. Cat component test PCR-test strip method;
- Part 2. Detection of mink composition by PCR-test strip method;
- Part 3. Detection of deer components by PCR-test strip method;

- Part 4. Detection of camel composition by PCR-test strip method;
- Part 5. Detection of dog ingredients by PCR-test strip method;
- Part 6. Detection of bovine constituents by PCR-test strip method;
- Part 7. Detection of sheep composition by PCR-test strip method;
- Part 8. Detection of donkey components by PCR-test strip method;
- Part 9. Detection of fox composition by PCR-test strip method;
- Part 10. Detection of goose composition by PCR-test strip method;
- Part 11. Detection of duck composition by PCR-test strip method;
- Part 12. Detection of turkey composition by PCR-test strip method;
- Part 13. Pigeon composition detection PCR-test strip method;

This part is the first part of Sn/T 5145.

This section is drafted in accordance with the rules given in GB/T 1.1-2009.

Please note that some elements of this document may involve patents. The issuer of this document is not responsible for identifying these patents.

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

This section was drafted. Tianjin Customs of the People's Republic of China, Tianjin Normal University, Beijing Customs of the People's Republic of China, Beijing Huada Egg White Matter R & D Center Co., Ltd.

1 Scope

This section specifies the PCR-test strip method for detecting cat-derived components in food and feed.

This section applies to the qualitative detection of cat-derived components in meat, meat products and feed.

2 Normative references

The following documents are essential for the application of this document. For dated references, only the dated version applies to this document.

file. For undated references, the latest version (including all amendments) applies to this document.

GB/T 6682 Analytical laboratory water specifications and test methods GB/T 14699.1 Feed

sampling GB/T 27403-2008 Laboratory quality control specifications Food molecular biological testing

3.1 Terms and definitions

The following terms and definitions apply to this document.

3.1.1

It belongs to the mammals, Carnivora, feline,. The cat component in this standard refers to cat-specific DNA fragments.

3.2 Abbreviations

The following abbreviations apply to this document.

DNA. deoxyribonucleic acid, DNA.

dNTP. deoxyribonucleoside triphosphate, deoxynucleotide triphosphate.

CTAB. cetyltrimethylammonium bromide, cetyltrimethylammonium bromide.

Tris. Tris (Hydroxymethyl) aminomethane, trimethylolaminomethane.

EDTA. Ethylenediacetic acid, ethylenediamine tetraacetic acid.

FITC. Fluorescein 5-isothiocyanate, isothiocyanate.

4 Method principle

Sample cells were disrupted with lysis buffer, genomic DNA was extracted by phenol-chloroform method, and the extracted DNA was used as a template, using upstream and downstream primers, the primers were amplified by PCR using isothiocyanate (FITC) and biotin (Biotin) markers, respectively.

PCR products with isothiocyanate and biotin molecules were obtained at both ends. Rabbit polyclonal antibody containing gold-labeled anti-isothiocyanate SN/T 514.5.1-2019
Screening and detection of PCR products, and sequencing of PCR-positive products
Confirmation.

5 Reagents and materials

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

5.1

The primer (pair) sequences and markers for detecting cat-derived components are.

Cat component amplification primers are shown in Table 1.

Table 1 Primers for testing

Species name primer sequence (5' → 3') 5' end marker target gene Cat F.

5'-CCATACATACGGGAGAC-3G isothiocyanate (FITC) R.

5'-TGAGGTGTTAAAAACATACTGAGATAGAGA-3A biotin CYTB

5.2 Reagents

5.2.1

ExTaqHotStatDNA polymerase (Taq, Termusauticu).

5.2.2 DNTPs. dATP (deoxyadenosophate, deoxyadenosine triphosphate), dTTP (deoxythymine)

triphosphatate (deoxythymidine triphosphate), dctp (deoxadenine cyclotin phosphate)
dGTP (deoxiy nooninphosphate, deoxyguanosine triphosphate).

5.2.3 10 x PCR buffer..200 mmol/L Tris-HC1 (pH 8.4),.200 mmol/LKCl, 20 mmol/LMGCl2.

5.2.4 Trichloromethane (chloroform).

5.2.5 Isopropanol.

5.2.6 CTAB extraction solution. 20g/LCTAB, 1.4mol/LNaCl, 0.1mol/LTris-HC1, 0.02mol/LNA2EDTA, pH

5.2.7 CTAB precipitation solution. 5g/LCTAB, 40mmol/LnaCl, pH 8.0.

5.2.8 Phenol/trichloromethane/isoamyl alcohol solution. According to Tris saturated phenol. trichloromethane. isoamyl alcohol solution volume ratio of 25. 24. 1 Preparation.

5.2.9 Trichloromethane/isoamyl alcohol solution. Prepare according to the volume ratio of trichloromethane. isoamyl alcohol solution is 24. 1.

5.2.10 70% ethanol (V/V).

5.2.11 NaCl solution (1.2mol/L).

5.2.12 Proteinase K (20mg/mL).

5.2.13 Test strips. a rabbit polyclonal antibody containing gold-labeled anti-isothiocyanate and a universal nucleic acid test strip immobilized with streptavidin Strips can be assembled by themselves using purchased raw materials (see Appendix A) or equivalent commercial test strips.

5.2.14 Developing solution. 10 mmol/L Tris, 1% BSA, 1% (v/v) Tween 20, and NaOH at a concentration of 0.05 mmol/L; or Use equivalent commercial products.