

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

SN/T 5145.7-2019

**Rapid Identification of animal ingredient in export food and feed
-- Part 7: Detection of Ovis aries Ingredient PCR-Lateral flow
dipstick method
(English Translation)**

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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 section is Part 7 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, Xining Customs of the People's Republic of China, Beijing Huada Egg White Matter R & D Center Co., Ltd., Tianjin University of Science and Technology.

1 Scope

This section specifies the PCR-test strip method for the detection of sheep components in food and feed.

This section applies to the qualitative detection of sheep 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, Artiodactyla, Bovine, Sheep. The sheep component in this standard refers to sheep-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 5145.7-2019
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

Primer (pair) sequences and markers for detecting sheep-derived components are.

See Table 1 for sheep component amplification primers.

Table 1 Primers for testing

Species name primer sequence (5' → 3') 5' end marker target gene Sheep F.
5'-TCCATATATTGTGCACACAAC-3C

5.2 Reagents

5.2.1

ExTaqHotStatDNA polymerase (Taq, *Thermusausticu*).

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-HCl (pH 8.4),.200 mmol/LKCl, 20 mmol/LMgCl₂.

5.2.4 Trichloromethane (chloroform).

5.2.5 Isopropanol.

5.2.6 CTAB extract solution. 20g/LCTAB, 1.4mol/LNaCl, 0.1mol/LTris-HCl,
0.02mol/LNA₂EDTA, pH 8.0.

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

5.2.8 Phenol/trichloromethane/isoamyl alcohol solution. The volume ratio of Tris saturated
phenol. chloroform. isoamyl alcohol solution is 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.