

SN

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

SN/T 2106-2008

**Determination of methylisothiazolinone and
chloromethylisothiazolinone in cosmetics for import and
export. Liquid chromatography
(English Translation)**

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1 Scope

This standard specifies the PCR method for the detection of chicken components in animal derived products.

This standard applies to the qualitative detection of chicken components in animal derived products.

2 Normative references

The following documents are essential to the application of this document. For the dated documents, only the versions with the dates indicated are applicable to this document; for the undated documents, only the latest version (including all the amendments) is applicable to this standard.

GB 4789.1 National food safety standard - Food microbiological examination - General guidelines

GB/T 5009.1 Methods of food hygienic analysis - Physical and chemical section - General principles

GB/T 6682 Water for analytical laboratory use - Specification and test methods

GB/T 14699.1 Feeding Stuffs - Sampling

SN/T 1193 General requirements for the laboratories for gene detection and identification

3 Reagents and materials

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

3.1

Primer (pair) sequence for detection of chicken-derived components:

Upstream: 5'-ctataatcgataatccacgattca-3' shake to mix it uniformly from time to time. Centrifuge it at 12000 r/min for 5 min. Transfer the supernatant into a clean centrifuge tube. Add 400 μ L of chloroform/isoamyl alcohol (24:1). Mix well. Centrifuge it at 12000 r/min for 5 min. Take the supernatant. Add 0.8 times the volume of isopropanol. Precipitate it. Centrifuge it at 12000 r/min for 5 min. Discard the supernatant. Use the 75% ethanol to rinse it once. Dry it naturally. Add 50 μ L of double-distilled water (ddH₂O), to dissolve the precipitate, to make a DNA solution. Prepare for use.

It may also use the equivalent DNA extraction kit, to extract sample DNA.

6.2 PCR amplification

Reaction system: The volume is 25 μ L, including 12.5 μ L of 2 X PCR buffer, 5

μ L of dNTPs (2 mmol/L), 0.5 μ L of primer pair (10 nmol/L), 1 μ L of DNA polymerase (1 U/ μ L), 5 μ L of template DNA (100 ng $\pm$ 50 ng DNA), 0.5 μ L of double-distilled water.

Reaction conditions: PCR reaction conditions vary slightly with different PCR instruments. Perform pre-denaturation at 94 °C for 1 min ~ 3 min, denaturation at 94 °C for 30 s ~ 60 s, annealing at 63 °C for 30 s ~ 60 s, extension at 72 °C for 30 s ~ 60 s, in a total of 35 cycles; perform extension at 72 °C for 5 min;

store it at 4 °C.

In the detection process, set a positive control, a negative control, a blank control, respectively.

6.3 Electrophoresis detection of the amplified product of PCR

Weigh 1.5 g of agarose in 100 mL of electrophoresis buffer. Heat it to fully melt.

Add ethidium bromide to a final concentration of 0.5 μ g/mL. Prepare a gel. Add the electrophoresis buffer to the electrophoresis tank, so that the liquid level has just covered the gel. Mix 8 μ L of each amplified product of PCR with 3 μ L of sample-added buffer. Apply the sample. Keep constant voltage at 9 V/cm.

Carry out electrophoresis, until the bromophenol blue indicator migrates to the middle of the gel. Observe and record the electrophoresis results under the gel imager.

6.4 Sequencing

When the electrophoresis result is suspiciously positive, the PCR amplification product is subjected to DNA sequencing.

7.1 Electrophoresis and sequence analysis of PCR amplification product

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