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NATIONAL STANDARD OF THE PEOPLE'S REPUBLIC OF CHINA

GB/T 20190-2025

Replacing GB/T 20190-2006

Determination of bovine, ovine and goat-derived materials in feeds

饲料中牛、绵羊和山羊源性成分的测定

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1 Scope

This document describes methods for the determination of bovine, ovine and goat- derived materials in feeds using real-time fluorescent polymerase chain reaction and polymerase chain reaction.

This document applies to the detection of bovine, ovine and goat-derived materials in compound feeds, concentrated feeds, concentrate supplements, feed ingredients, compound premixes and mixed feed additives.

The detection limit for this document is 0.1%.

2 Normative references

GB/T 6682

GB/T 19495.3-2004

GB/T 20195

GB/T 27403-2008

GB/T 35918-2018

3 Terms and definitions

No terms and definitions need to be defined in this document.

4 Abbreviated terms

For the purposes of this document, the following abbreviated terms apply. bp. base pair Ct. cycle threshold CTAB. cetyltrimethylammonium bromide DNA. deoxyribonucleic acid EDTA. ethylene diamine tetraacetic acid PCR. polymerase chain reaction Tris. trihydroxymethyl aminomethane

5.1 Principle

Design primers and probes based on the specific sequences of the bovine beta-actin gene, the ovine prolactin receptor gene, and the goat chromosome 9 gene. Use real-time fluorescence PCR technology for amplification, and detect the bovine, ovine, and goat-derived DNA components based on the fluorescent signal and Ct value generated in the amplification reaction.

5.2 Reagents or materials

Unless otherwise specified, use analytical reagents only. All reagents shall be stored or aliquoted in containers free of DNA enzyme contamination.

5.2.1 Water. GB/T 6682, grade 1.

5.2.2 2x real-time PCR premix. Containing Taq DNA polymerase, real-time fluorescence PCR buffer, MgCl2, and dNTPs; shall not contain bovine serum albumin.

5.2.3 Sodium hydroxide solution (10 mol/L). Weigh 400 g of sodium hydroxide; add

water to fix the volume to 1 L.

5.2.4 Tris-hydrochloric acid solution (1 mol/L). Weigh 121.1 g of Tris and dissolve it in 800 mL of water; use hydrochloric acid to adjust the pH to 8.0; add water to dilute to 1 L. Sterilize at 103.4 kPa and 121 °C for 20 min and store at room temperature.

5.2.5 EDTA solution (0.5 mol/L). Weigh 186.1 g of Na2EDTA-2H2O and dissolve it in 800 mL of water; stir vigorously on a magnetic stirrer; add approximately 20 g of 5.3.2 Analytical balance. precision 0.1 mg.

5.3.3 Centrifuge. centrifugal speed not less than 14 000 r/min.

5.3.4 Ultraviolet spectrophotometer or micro-nucleic acid protein analyzer.

5.3.5 Micropipettes. 2.5 µL, 10 µL, 100 µL, 200 µL, 1 000 µL, etc.

5.4 Test sample

Prepare a sample of at least 200 g according to GB/T 20195; crush or grind it so that it passes through a 0.25 mm test sieve; mix it thoroughly; place it in a sealed container for later use. Other crushing or grinding methods that have been shown to be equivalent may also be used.

Prepare the positive control sample (5.2.8) and negative control sample (5.2.9) in the same manner and set aside.

Note. After crushing or grinding a sample, clean the crusher or mill container and knives to prevent contamination.

5.5.1 DNA extraction

Perform two tests in parallel. Weigh 100 mg ~ 200 mg of the sample; extract DNA according to the method in C.6.2 of Appendix C of GB/T 19495.3-2004.Alternatively, use a verified and reliable DNA extraction kit for DNA extraction. Refer to the instructions for specific use. The extraction of blank control is the same as that of sample except that no sample is added. If necessary, extract DNA from positive and negative control samples. If the extracted DNA needs to be stored, store it below -18°C.

5.5.2 DNA solution concentration determination

Measure the concentration of the DNA solution (5.5.1) using an ultraviolet spectrophotometer (wavelength. 260 nm) or a micro-nucleic acid protein analyzer (5.3.4). The mass concentration of DNA solution should be controlled at 5 ng/μL ~ 50 ng/μL.

5.5.3 Control settings

During the real-time fluorescence detection process, positive controls, negative controls, amplification blank controls, and extraction blank controls shall be set up (5.5.1). Use the DNA from the positive sample (5.2.8) as the positive control, and the DNA from the negative sample (5.2.9) as the negative control. Use an equal volume of water to replace the DNA solution as the amplification blank control.

5.6.2 Amplification of bovine, ovine and goat-derived specific genes

Real-time fluorescence PCR detection of bovine, ovine and goat-derived specific genes shall meet the following conditions.

a) Extraction of blank control. Ct value = 40.0 or no Ct value;

b) Amplification of blank control. Ct value = 40.0 or no Ct value;

c) Negative control. Ct value = 40.0 or no Ct value;

d) Positive control. Fluorescent signal is detected in the fluorescence channel and a typical amplification curve appears, with a Ct value <= 35.0.

5.7.1 Result judgment

When compliance with 5.6 is achieved, the test results of the test samples shall be determined as follows.

a) If the Ct value is <=35.0, the test sample is considered positive;

b) If the Ct value is =40.0 or there is no Ct value, the sample is considered negative;

c) If 35.0< Ct value< 40.0, re-measurement is required. If the Ct value after further amplification is still less than 40.0, the sample is judged to be positive; if the Ct value after further amplification is = 40 or there is no Ct value, the sample is judged to be negative.

5.7.2 Result expression

A positive result is expressed as "xx-derived DNA component detected".

A negative result is expressed as "no xx-derived DNA component detected".

6.1 Principle

Design primers based on the specific sequences of mitochondrial genes of bovine, ovine and goat, and use PCR technology for amplification. Separate PCR products by electrophoresis; compare with DNA molecular weight markers; sequence, and compare with species reference sequences in the gene library to detect DNA components derived from bovine, ovine, and goat.

6.2 Reagents and materials

Unless otherwise specified, use analytical reagents only. All reagents shall be stored or aliquoted in containers free of DNA enzyme contamination.

6.2.1 Water. GB/T 6682, grade 1. 6.2.2 n-Hexane. 6.2.3 Isopropyl alcohol.

6.2.4 2x PCR reaction premix. Containing Taq DNA polymerase, PCR buffer, MgCl2 and dNTPs; shall not contain bovine serum albumin.

6.2.5 Ethanol with a volume fraction of 75%. 6.2.6 Agarose.

6.2.7 Nucleic acid dye Goldview solution (10 000x).

6.2.8 DNA molecular weight markers (covering the range of 100 bp ~ 500 bp).

6.2.9 PCR product recovery and purification kit. follow the instructions.

6.2.10 DNA sequencing reagents.