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

GB/T 20191-2025

Replacing GB/T 20191-2006

**Microbiological examination of Lactobacillus acidophilus in
feeds**

饲料中嗜酸乳杆菌的微生物学检验

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Foreword

This document complies with the provisions of GB/T 1.1-2020 "Standardization Work Guidelines Part

1. Structure and Drafting Rules of Standardization Documents". Drafting. This document supersedes GB/T 20191-2006 "Microbiological Examination of Lactobacillus Acidophilus in Feed" and is consistent with GB/T 20191-2006. Compared to previous versions, aside from structural adjustments and editorial changes, the main technical changes are as follows:

- a) The scope of application has been changed (see Chapter 1, Chapter 1 of the.2006 edition);
 - b) The sample preparation method has been changed (see 7.1, 6.2.1 of the.2006 edition);
 - c) The culture conditions and culture time for Lactobacillus acidophilus were changed (see 7.3, 6.2.6 of the.2006 edition);
 - d) The experimental data processing method was changed (see Chapter 8, Chapter 8 of the.2006 edition);
 - e) The type and preparation method of the Lactobacillus acidophilus culture medium were changed (see A.1,.2006 edition of A.1);
 - f) Added molecular biological identification methods for Lactobacillus acidophilus (see B.2).
- Please note that some content in this document may involve patents. The issuing organization of this document assumes no responsibility for identifying patents. This document was proposed and is under the jurisdiction of the National Technical Committee on Standardization of Feed Industry (SAC/TC76). This document was drafted by: Hubei Jingrui Tianheng Biotechnology Co., Ltd., Beijing Institute of Animal Husbandry and Veterinary Medicine, Chinese Academy of Agricultural Sciences, and Hubei Kai Neng

Biotechnology Co., Ltd., Beijing Academy of Agricultural and Forestry Sciences, Hubei Provincial Institute of Veterinary Drug Inspection, National Center for Food Safety Risk Assessment, Beijing Dabeinong Technology Group Co., Ltd., and Beijing Newtech Open Source Gene Technology Co., Ltd. The main drafters of this document are: Chen Xiaofeng, Rao Zhenghua, Xu Pengpeng, Zhang Dongyan, Wen Jingjing, Han Xiaomin, Liu Xuelian, Wang Jianhua, Wang Jun, and Lin Yan. Liu Ying, Bie Junyan, Liang Mingyuan, Liu Na. The release history of this document and the document it replaces is as follows:

---First published in 2006 as GB/T 20191-2006;

---This is the first revision. Microbiological testing of *Lactobacillus acidophilus* in feed

1 Scope

GB/T 20191-2025 is the Chinese national standard covering counting the probiotic in a feed - the viable count of *Lactobacillus acidophilus*, which is what the additive is sold on and which falls during pelleting and storage. It replaces GB/T 20191-2006. It was issued on 31 December 2025 and has been in force since 1 July 2026, replacing GB/T 20191-2006. The document is under the responsibility of the Standardization Administration of China. This page is published from the official record of the 2025 edition; the clause text of a standard this recent is not yet in circulation, and the figures, limits and tables it contains are those of the document itself, delivered in full with the English translation.

This document applies to the counting and identification of the total number of viable *Lactobacillus acidophilus* in feed containing *Lactobacillus acidophilus*.

2 Normative references

The contents of the following documents, through normative references within the text, constitute essential provisions of this document. Dated citations are not included. For references to documents, only the version corresponding to that date applies to this document; for undated references, the latest version (including all amendments) applies. This document.

GB/T 6682 Specifications and test methods for water used in analytical laboratories

GB/T 30989-2014 Technical Specifications for High-Throughput Gene Sequencing

GB/T 40664-2021 General requirements for quality control of nucleic acid samples used in high-throughput sequencing

GB/T 42959 Sample collection for microbiological testing of feed

3 Terms and Definitions

This document does not contain any terms or definitions that need to be defined.

4. Reagents and Culture Media Unless otherwise specified, use only analytical grade reagents.

4.1 Water. GB/T 6682, Grade III.

4.2 MRS agar medium. Prepared according to Appendix A, section A.1.

4.3 Lactobacillus sugar fermentation tubes. Prepared according to A.2.

4.4 Aescin medium. Prepared according to A.3.

4.5 Gram staining solution. Prepared according to A.4.

4.6 Physiological saline. Prepared according to A.5.

5 Equipment and Materials

5.1 Incubator. $36^{\circ}\text{C} \pm 1^{\circ}\text{C}$.

5.2 Balance. sensitivity 0.1g and 0.01g.

5.3 Thermostatic oscillator.

5.4 Vortex mixer.

5.5 Sterile petri dishes. 90 mm in diameter.

5.6 Anaerobic culture apparatus. anaerobic incubator, anaerobic tank, anaerobic bag or apparatus that can provide equivalent anaerobic effects.

5.7 Sterile pipettes. pipettes with a capacity of

0.1 mL, 1 mL or equivalent, and matching sterile pipette tips.