

ICS 65.120

CCS B 46



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

GB/T 44738-2024

Determination of feed pectinase activity

饲用果胶酶活力的测定

Issued on: October 26, 2024

Implemented on: May 1, 2025

**Issued by: State Administration for Market Regulation;
Standardization Administration of the PRC**

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Foreword

This document is in accordance with the provisions of GB/T 1.1-2020 "Guidelines for standardization work Part

1. Structure and drafting rules for standardization documents" Drafting is required. Please note that some of the contents of this document may involve patents. The issuing organization of this document does not assume the responsibility for identifying patents. This document is proposed and coordinated by the National Feed Industry Standardization Technical Committee (SAC/TC 76). This document was drafted by: Wuhan Xinhuiyang Biotechnology Co., Ltd., Beijing Xindayang Technology Development Co., Ltd., Guangdong Yidoli Biotechnology Co., Ltd. Technology Co., Ltd., Shandong Longkote Enzyme Preparation Co., Ltd., and Novozymes (China) Investment Co., Ltd. The main drafters of this document are: Zhou Ying, Xu Li, Zhan Zhichun, Zhao Lifang, Feng Guohua, Zhang Faling, Sun Xuwei, Sudan, Yu Yan, Li Yangyuan, Wang Kefen and Wang Jinfeng. Determination of feed pectinase activity

1 Scope

China's national method for determining pectinase activity in feed. Pectin is a structural polysaccharide of plant cell walls, abundant in the by-products that make up much of a modern feed ration - sugar beet pulp, citrus pulp, rapeseed and sunflower meals - and monogastric animals produce no enzyme that breaks it down. Undigested pectin does two things, both unhelpful: it is energy the animal paid for and cannot use, and in solution it raises the viscosity of the gut contents, which slows the digestion of everything else and produces the wet litter that is a persistent problem in poultry houses. Added pectinase addresses both. Since the enzyme is dosed in grams per tonne and sold on activity, the assay is what the trade rests on, and it must be run under conditions representing the gut rather than the optimum for the enzyme, which is where such methods diverge.

This document describes a method for the spectrophotometric determination of the activity of feed pectinase. This document is applicable to the determination of pectinase activity in feed additive pectinase and mixed feed additive enzyme preparations containing pectinase. This document is not applicable to the determination of alkaline pectinase activity. The limit of quantification of this method is 10 U/g (or U/mL).

2 Normative references

The contents of the following documents constitute the essential clauses of this document through normative references in this document. For referenced documents without a date, only the latest version (including all amendments) applies to this document. In this document.

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

GB/T 20195 Preparation of animal feed samples

3 Terms and Definitions

The following terms and definitions apply to this document.

3.1 Pectinase activity unit At 37 °C and pH 3.5, the amount of reducing sugar released from a pectin solution with a mass concentration of

2.25 mg/mL per hour is The amount of enzyme required.

Note. Indicated by U.

4 Principles

Pectinase hydrolyzes pectin solution to produce reducing sugar, which is then developed with 3,5-dinitrosalicylic acid and measured with a spectrophotometer to calculate the pectin

content. Enzyme activity.

5 Reagents or materials

Unless otherwise stated, only analytical grade reagents were used.

5.1 Water. GB/T 6682, Grade III.

5.2 Sodium hydroxide solution (200 g/L). Weigh

20.0 g of sodium hydroxide, dissolve it in water, cool to room temperature, and then dilute to 100 mL.

5.3 Citric acid solution (

0.1 mol/L). Weigh

21.01 g of citric acid monohydrate, dissolve it in water and make up to 1000 mL.

5.4 Trisodium citrate solution (

0.1 mol/L). Weigh

29.41 g of trisodium citrate dihydrate, dissolve it in water and make up to 1000 mL.

5.5 Citric acidsodium citrate buffer (

0.1 mol/L, pH=3.5). Take 700 mL of citric acid solution (5.3), trisodium citrate solution Add 300 mL of solution (5.4), mix well, and adjust the pH to

3.5 with citric acid solution (5.3) or trisodium citrate solution (5.4).

5.6 Pectin solution (

3.0 mg/mL). Weigh

0.3 g of pectin ($\geq 74\%$ polygalacturonic acid, CAS No.. 9000695) and