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NATIONAL STANDARD OF THE PEOPLE'S REPUBLIC OF CHINA

GB/T 47201-2026

Assay method for calf intestinal alkaline phosphatase activity and purity

小牛肠碱性磷酸酶活性及纯度检测方法

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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. 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 Working Group on Standardization of Tool Enzymes (SAC/SWG11). This document was drafted by: Nansheng (Xiamen) Analytical Testing Co., Ltd., Shenya Biotechnology Co., Ltd., and Xiahe (Shenzhen) Biotechnology Co., Ltd. Technology Co., Ltd., Beijing Solarbio Technology Co., Ltd., Xiahe (Guangzhou) Optoelectronic Biotechnology Co., Ltd., Wuhan Xinhua Yang Biotechnology Co., Ltd. The company, Hangzhou Boyue Biotechnology Co., Ltd., Guangzhou Wondfo Biotech Co., Ltd., Jiangsu Chuangjian Medical Technology Co., Ltd. Beijing Leadman Biochemical Co., Ltd., Fujian Saif Safety Technology Service Co., Ltd., Xiahe (Hangzhou) Biotechnology Co., Ltd., Huacan (Xiamen) Biomedical Co., Ltd., Fujian Nansheng Technology Co., Ltd., Datian Huacan Biotechnology Co., Ltd., Hainan Huayan Collagen Technology Co., Ltd. Limited Liability Company, Shandong University, Soochow University, Shanghai Jiao Tong University, Fudan University, Fujian Agriculture and Forestry University, Zhejiang University of Commerce and Industry, Zhejiang Normal University School, Xiamen University, Shanghai Chuyu Biotechnology Co., Ltd. The main drafters of this document are. Huang Facan, Liu Hongyan, Huang Enming, Ma Yuling, Zheng Dengzhong, Xu Li, Li Yinlai, Sun Yaling, Fan Xiaoju, and Li Ran. Chen Yaping, Guo Yanwei, Pan Wei, Wang Fang, Hu Yue, Zhao Zifang, Fu Linglin, Chen Xiulan, Feng Yan, Zhao Chao, Wang Cui, Shen Tao, Yang Zhonghua, Liu Bin Xing Zhigang, Zhong Jiang, Zhu Li, Yao Juan, Zhang Yongyou, Zheng Tianye, Huang Sitao, Pan Paifeng, Huang Haiyan, Zheng Liming, Huang Lianhua.

Calf intestinal alkaline phosphatase is a highly efficient catalyst that catalyzes the

dephosphorylation of 5' and 3' phosphate monoesters and hydrolyzes them to release 5'-phosphate monoesters under alkaline conditions. Hydrolases with terminal or 3' phosphate groups are used for antibody or antigen labeling. A method for detecting the activity and purity of calf intestinal alkaline phosphatase was developed. National standards for these laws are used to promote production and use, and are of great significance for promoting the industrialization of this type of tool enzyme. Methods for detecting the activity and purity of calf intestinal alkaline phosphatase

1 Scope

GB/T 47201-2026 is the Chinese national standard covering assaying calf intestinal alkaline phosphatase - the enzyme used to dephosphorylate DNA in cloning and as a label in immunoassays, where contaminating nucleases are as important as the stated activity. First edition, in force since 1 June 2026. It was issued on 27 February 2026 and has been in force since 1 June 2026, as a first edition. The document is under the responsibility of the Standardization Administration of China. This page is published from the official record of the 2026 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 describes a method for detecting the activity and purity of calf intestinal alkaline phosphatase. This document applies to the detection of the activity and purity of calf intestinal alkaline phosphatase.

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 40174-2021 Methods for Determination of Purity of Tool Enzymes

3 Terms and Definitions

The following terms and definitions apply to this document.

3.1 A hydrolase that can efficiently catalyze the dephosphorylation of 5' and 3' phosphate monoesters under alkaline conditions.

3.2 In diethanolamine (DEA) buffer at pH 9.8, at 37°C, calf intestinal alkaline phosphatase hydrolyzed p-nitrophenyl phosphate per minute. The amount of enzyme required to produce

1 μmol of p-nitrophenol (PNP) from the ester (PNPP) chromogenic substrate is one active unit.

4.Principles Using p-nitrophenyl phosphate (PNPP) as a substrate and diethanolamine as the phosphate acceptor, alkaline phosphate in calf intestines is synthesized under alkaline conditions. Enzymatic hydrolysis of PNPP produces free yellow p-nitrophenol (PNP), which exhibits a specific absorption peak at a wavelength of 405 nm. The activity units of the enzyme were calculated by measuring the rate of increase in absorbance at 405 nm.

5 Reagents or materials

5.1 Water Water that meets the requirements of GB/T 6682 (Grade II). 5.2 1 mol/L magnesium chloride solution Weigh

20.33 g of magnesium chloride hexahydrate (analytical grade), dissolve it in water, and dilute to 100 mL.