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

GB/T 46389-2025

Determination of the activity of heparin lyase

肝素酶活力的测定

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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 publishing 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 Biochemical Testing (SAC/TC387). This document was drafted by: Tsinghua University, Hebei Provincial Institute of Food Inspection and Testing, China Academy of Testing Technology, and Tsinghua University Shenzhen International Research Center. Life Sciences Institute, Suzhou Shipu Testing Technology Co., Ltd., Tsinghua University Beijing Tsinghua Chang Gung Hospital, Soochow University, Shenzhen Second People's Hospital, China China Association of Biochemical Pharmaceutical Industries, China Association of Biofermentation Industry, China Metrology and Testing Society. The main drafters of this document are. Wang Yi, Xing Xinhui, Zhang Yan, Zhou Lihua, Zhang Chong, Zhang Weiwei, Su Nan, Lu Jian, Jiang Xuan, Gu Dayong, and Wang Xu. Li Weifeng, Zhang Zhenqing, Xu Bing, Yang Yangzhongfu, Hu Wenyan, Zhou Bin, Wang Jin, Wang Jie. Assay for heparinase activity

1 Scope

GB/T 46389-2025 is the Chinese national standard covering measuring heparinase activity - the substrate and the buffer, the increase in absorbance as the enzyme cuts the glycosaminoglycan chain, the unit definition, and the conditions that have to be held for the figure to be comparable between suppliers. The enzyme is used both in heparin manufacture and in the assay of it. First edition, in force from 1 May 2026. Issued on 5 October 2025, it has been in force since 1 May 2026.

This document describes a spectrophotometric method for determining heparinase activity. This document applies to the determination of the activities of heparinase I, heparinase II, and heparinase III in biochemical reagents.

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

3 Terms and Definitions

The following terms and definitions apply to this document. 3.1 1000 μL of substrate solution (5.2.2, 5.2.3, 5.2.4) at 30°C and pH

7.0 produces 1 μmol of 4,5-unsaturated uronic acid per minute through enzymatic hydrolysis. The amount of enzyme required for saturated uronic acid.

Note. Enzyme activity is expressed in IU. 1 IU = 1.667×10^{-8} mol/s.

4. Principles Heparinase catalyzes the cleavage of substrates such as sodium heparin or heparan sulfate, producing the cleavage product 4,5-unsaturated uronic acid. Aldol acids exhibit a characteristic absorption peak at 232 nm. By measuring the absorbance change at 232 nm, and combining 4,5-unsaturated uronic acid... Calculate enzyme activity using the molar extinction coefficient at 232 nm.

5. Reagents and Materials Unless otherwise stated, all reagents used in this method are of analytical grade, and the water is grade II water as specified in GB/T 6682.

5.1 Reagents

5.1.1 Sodium heparin (CAS No. 9041-08-1). purity $\geq 99\%$, or a standard substance/standard sample that has been certified by the state and granted a certificate.

5.1.2 Heparin sulfate (CAS No. 9050-30-0). Purity $\geq 99\%$, or a nationally certified reference material/standard. sample.

5.1.3 Hydrochloric acid (HCl).

5.1.4 Tris(hydroxymethyl)aminomethane (Tris).

5.1.5 Calcium chloride (CaCl_2).