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

GB/T 46387-2025

Determination of the activity of chondroitinase

硫酸软骨素酶活力的测定

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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 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 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, Beijing Yanwei Technology Co., Ltd., Suzhou Shipu Testing Technology Co., Ltd., Tsinghua University Beijing Tsinghua Changgeng Hospital, Soochow University, Shenzhen Shenzhen Second People's Hospital, China Biochemical Pharmaceutical Industry Association, China Biofermentation Industry Association, 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, Wang Miaomiao, Lu Jian, Du Yan, and Jiang Xuan. Gu Dayong, Wang Xu, Li Weifeng, Zhang Zhenqing, Xu Bing, Yang Yangzhongfu, Hu Wenyan, Zhou Bin, Wang Jin, Wang Jie. Assay for chondroitin sulfate activity

1 Scope

GB/T 46387-2025 is the Chinese national standard covering chondroitinase activity - the substrate, the reaction conditions and the spectrophotometric measurement of the unsaturated product, the unit definition and the calculation, on an enzyme used in the analysis and the production of chondroitin sulfate. First edition, with the heparin lyase method GB/T 46389-2025. 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 chondroitin sulfate activity. This document applies to the determination of the activities of chondroitin sulfate

AC and chondroitin sulfate B using 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) at 30 °C and pH

7.5 produces 1 μmol of 4,5-unsaturated sugar per minute through enzymatic hydrolysis. The amount of enzyme required for aldehyde acid production.

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

4.Principles Chondroitin sulfate enzymes catalyze the cleavage of substrates such as sodium chondroitin sulfate or dermatan sulfate, producing the cleavage product 4,5-unsaturated uronic acid. 4,5-Unsaturated uronic acid exhibits a characteristic absorption peak at 232 nm. By measuring the absorbance change at 232 nm, and considering the 4,5-unsaturated... The enzyme activity was calculated by taking the molar extinction coefficient of uronic acid 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 Chondroitin sulfate sodium (CAS No. 9082-07-9). purity $\geq 99\%$, or a standard substance/standard certified by the state and granted a certificate. sample.

5.1.2 Dermatin sulfate (CAS No. 54328-33-5). Purity $\geq 99\%$, or a nationally certified reference material/standard. sample.

5.1.3 Hydrochloric acid (HCl).

5.1.4 Tris(hydroxymethyl)aminomethane (Tris).