

ICS 07.080
CCS A 40



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

GB/T 46452-2025

Purity and enzymatic activity determination of nitrile hydratase

腈水合酶纯度和活性的测定

Issued on: October 5, 2025

Implemented on: February 1, 2026

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

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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: Shenzhen Second People's Hospital, Jiangnan University, Shenzhen Mindray Bio-Medical Electronics Co., Ltd., and China Testing. Technology Research Institute, Beijing Yanwei Technology Co., Ltd., Tsinghua University, Hebei Provincial Institute for Food Inspection and Testing, China Metrology and Testing Society. The main drafters of this document are: Gu Dayong, Cui Wenjing, Zhou Zhemin, Li Weifeng, Zhang Yiwen, Zhou Lihua, Wang Miaomiao, Li Fulong, Wang Yi, and Zhang Chong. Zhang Yan, Wang Xu, and Yang Yangzhongfu. Determination of purity and activity of nitrile hydratase

1 Scope

GB/T 46452-2025 is the Chinese national standard covering assaying nitrile hydratase - the electrophoretic and chromatographic purity, the activity measured as acrylamide formed from acrylonitrile per unit time, the unit definition, and the conditions that have to be held for the figure to mean anything. This enzyme makes most of the world's acrylamide. First edition, in force from 1 February 2026. Issued on 5 October 2025, it has been in force since 1 February 2026.

This document describes the principle, reagents and materials, instruments and equipment, sample preparation, experimental procedures, and results for the determination of nitrile hydratase purity and activity. calculate. This document applies to the determination of the purity and activity of nitrile hydratases that catalyze the conversion of acrylonitrile to acrylamide.

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 Nitrile hydratase (NHase) Metalloenzymes that catalyze the conversion of nitrile substances (such as acrylonitrile) into amide compounds (such as acrylamide). Note

1. Most NHases contain two subunits, alpha and beta, and generally exist in the form of alpha₂beta₂ tetramer. Note

2. The active site of NHase contains different metal ions, and is generally divided into iron-type nitrile hydratase (Fe-NHase) and cobalt-type nitrile hydratase (Co-NHase).

3.2 Nitrile hydratase purity Relative purity of nitrile hydratase protein.

Note. This refers to the ratio of the gray value detected by nitrile hydratase to the total gray value of the sample protein, and the result is expressed as a percentage (%).

3.3 The ability of nitrile hydratase to catalyze the conversion of acrylonitrile to acrylamide.

Note. The specific activity of the enzyme is expressed in units (U/mg).

4. Abbreviations The following abbreviations apply to this document. Acr-Bis. Methylene bisacrylamide (acrylamide-bisacrylamide) BSA. Bovine serum albumin Nhase. Nitrile hydratase