

National Institute of Advanced Industrial Science and Technology  
National Metrology Institute of Japan



## Reference Material Certificate

NMIJ CRM 6024-b

No. +++

L-Histidine



This certified reference material (CRM) is produced in accordance with the NMIJ's management system and is in compliance with ISO 17034 and ISO/IEC 17025. This CRM is intended for the calibration of analytical instruments, preparation of standard solutions, and validation of analytical methods and instruments used for the amino acid analysis.

**Certified Values**

The certified value for the purity (in mass fraction) of L-histidine is given in the table below. The uncertainty of the certified value is the expanded uncertainty obtained by multiplying the combined standard uncertainty by a coverage factor ( $k$ ) of 2, and it is the half-width of an interval estimated to have a level of confidence of approximately 95 %.

Substance	CAS No.	Certified value Mass fraction (kg/kg)	Expanded uncertainty Mass fraction (kg/kg)
L-Histidine ( <i>(2S)</i> -2-amino-3-( <i>1H</i> -imidazol-5-yl) propanoic acid)	71-00-1	0.999	0.002

The purity (in mass fraction) of histidine without enantiomeric separation is given in the table below. The uncertainty of the certified value is the expanded uncertainty obtained by multiplying the combined standard uncertainty by a coverage factor ( $k$ ) of 2, and it is the half-width of an interval estimated to have a level of confidence of approximately 95 %.

	Certified value, Mass fraction (kg/kg)	Expanded uncertainty, Mass fraction (kg/kg)
Histidine (without enantiomeric separation)	0.999	0.002

**Analysis**

The certified value of this CRM was weighted mean of the results of the following analytical methods:

- (1) Acidimetric titration corrected the bias due to the amount of amino acid-related impurities
- (2) Nitrogen determination by Kjeldahl method corrected the bias due to the amount of amino acid-related impurities

Amino acid-related impurities were identified and quantified by HPLC with fluorescence detection after derivatization using *o*-phthalaldehyde (OPA). D-histidine was determined by the LC/MS using a chiral resolution column.

**Metrological Traceability**

The certified value was determined by titrimetry which was one of the primary method of measurement with NMIJ CRM 3001-c (potassium hydrogen phthalate) or NMIJ CRM 3012-a (tris(hydroxymethyl)aminomethane) and by amino acid-related impurity determination using HPLC calibrated with purity-evaluated amino acids. The certified values, therefore, is traceable to the International System of Units (SI).

**Mutual Recognition Arrangement under Metre Convention**

The certified value of this CRM without enantiomeric separation (mass fraction) is recognized for international equivalence based

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on the Mutual Recognition Arrangement under the Metre Convention (CIPM MRA). The calibration measurement capability (capabilities) (CMC) of NMIJ related to this CRM is registered in the Key Comparison Database (KCDB) (see <https://www.bipm.org/kcdb/>) of the International Bureau of Weights and Measures (BIPM).

#### **Expiration of Certification**

This certificate is valid for one year from the date of shipment, provided that this CRM is stored in accordance with the instructions given in this certificate.

#### **Description of the Material**

This CRM is in the form of a white powder of L-histidine and 1 g in net volume is kept in a brown glass vial and the vial is sealed in an aluminum-laminated plastic bag.

#### **Instructions for Storage**

This CRM should be stored at a temperature between 15 °C and 25 °C in a clean desiccator and shielded from light.

#### **Instructions for Use**

Considering the homogeneity, a minimum sample mass of 63 mg should be used. The CRM is for laboratory use only and not for *in vivo* use. The CRM should be used promptly once the vial is opened.

#### **Precautions for Handling**

Refer to the safety data sheet (SDS) on this CRM before use.

#### **Preparation**

Preparation of the material was performed by FUJIFILM Wako Pure Chemical Corporation. Highly purified L-histidine produced by AJINOMOTO Co., Inc. was bottled into vials under argon atmosphere. Each vial was sealed in an aluminum-laminated bag.

#### **Technical Information**

At the time of certification, more than 0.1 g/kg amino acid-related impurities were not detected. The molar mass of histidine used to calculate the certified value is 155.155 g/mol.

#### **NMIJ Analysts**

The technical manager for this CRM is KATO M., the production manager is MIYAMOTO A., and the analysts are MIYAMOTO A. and EYAMA S.

#### **Information**

If substantive technical changes occur that affect the certification before the expiration of this certificate, NMIJ will notify the registered customers. Customer registration on the NMIJ Website (given below) will facilitate notification. Technical reports regarding this CRM can be obtained from the contact details given below.

#### **Reproduction of Certificate**

In reproducing this certificate, it should be clearly indicated that the document is a copy.

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June 26, 2023

ISHIMURA Kazuhiko  
President  
National Institute of Advanced Industrial Science and Technology

If you have any questions about this CRM, please contact:  
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Sample