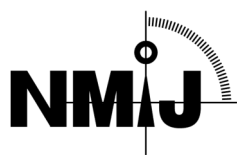


National Institute of Advanced Industrial Science and Technology

## National Metrology Institute of Japan



## Reference Material Certificate

NMIJ CRM 6012-a

No. +++)

L-Leucine



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. It is primarily intended for use in calibrating the analytical instruments or reagents in amino acid analysis. It is also intended for controlling the precision of analysis, and for confirming the validity of analytical methods or instruments.

**Certified Values**

The certified value for the purity (in mass fraction) of L-leucine is given in the table below. The uncertainty of the certified value is the half-width of the expanded uncertainty interval calculated using a coverage factor ( $k$ ) of 2, which gives a level of confidence of approximately 95 %.

	CAS No.	Certified value, Mass fraction (kg/kg)	Expanded uncertainty, Mass fraction (kg/kg)
L-Leucine ((2S)-2-amino-4-methyl pentanoic acid)	61-90-5	0.999	0.002

The certified value and expanded uncertainty of leucine without enantiomeric separation is given in the table below, where the mass fraction of D-leucine is negligible. The uncertainty of the certified value is the half-width of the expanded uncertainty interval calculated using a coverage factor ( $k$ ) of 2, which gives a level of confidence of approximately 95 %.

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

**Analysis**

The certified value is based on the results of non-aqueous acidimetric titration, nitrogen determination by the Kjeldahl method and impurity determination by high performance liquid chromatography. Impurities of amino acids were quantified by LC with fluorescence detection after derivatization using o-phthalaldehyde (OPA) and using 6-aminoquinolyl-N-hydroxysuccinimidyl carbamate (AQC). D-leucine was quantified by LC/MS using a chiral resolution column.

**Metrological Traceability**

The certified value was determined by titrimetry as the primary method of measurement with NMIJ CRM 3001-a (potassium hydrogen phthalate) and NMIJ CRM 3005-a (sodium carbonate) as primary standards and by impurity determination using HPLC calibrated with purity-evaluated amino acids. It is traceable to the International System of Units (SI).

**Mutual Recognition Arrangement under Meter Convention**

The certified value and expanded uncertainty of this CRM without enantiomeric separation (mass fraction) is consistent with the calibration and measurement capabilities (CMCs) that are included in Appendix C of the Mutual Recognition Arrangement (MRA) drawn up by the International Committee for Weights and Measures (CIPM). Under the MRA, all participating institutes recognize the validity of each other's calibration and measurement certificates for the quantities, ranges and measurement uncertainties specified in Appendix C (as for Appendix C of MRA, see <http://kcdb.bipm.org/AppendixC/default.asp>).

**Expiration of Certification**

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

**Sample Form**

This CRM is in the form of a white powder. This CRM of 0.5 g in net volume is kept in an amber glass vial and the vial is sealed in an aluminum-laminated plastic bag.

**Homogeneity**

The homogeneity of this CRM was measured by acidimetric titration, analyzing 10 vials randomly selected from 400 vials. The homogeneity is reflected in the uncertainty of the certified value.

**Instructions for Storage**

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

**Instruction for Use**

Considering the homogeneity, a minimum sample mass of 100 mg should be used. The CRM should be used promptly once a vial is opened. The CRM is for laboratory use only and not for in vivo use. Refer to the safety data sheet (SDS) on this CRM before use.

**Preparation**

Preparation of the material was performed by Wako Pure Chemical Industries, Ltd. Highly purified L-leucine provided by AJINOMOTO Co., Inc. was bottled into vials under argon atmosphere and 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.

**NMIJ Analysts**

The technical manager for this CRM is TAKATSU A. and production manager is KATO M. The analysts are KATO M., YAMAZAKI T., KATO H., EYAMA S., GOTO M. and YOSHIOKA M.

**Information**

If substantive technical changes occur that affect the certification before the expiration of this certificate, NMIJ will notify the registered customer. 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.

April 1, 2020

ISHIMURA Kazuhiko  
President

National Institute of Advanced Industrial Science and Technology

If you have any questions about this CRM, please contact:  
National Institute of Advanced Industrial Science and Technology,  
National Metrology Institute of Japan,  
Center for Quality Management of Metrology, Reference Materials Office,  
1-1-1 Umezono, Tsukuba, Ibaraki 305-8563, Japan  
Phone: +81-29-861-4059; Fax: +81-29-861-4009, <https://unit.aist.go.jp/nmij/english/refmate/>

Revision history

April 1, 2015: "Metrology Management Center" was renamed to "Center for Quality Management of Metrology."

April 11, 2017 The description on "Precautions for Handling" was added.

Jan. 24, 2019: The description on "Mutual Recognition Arrangement under Meter Convention" was added.

Sep. 19, 2019 The description in "Expiration of Certification" was changed to "one year from the date of shipment."