National Institute of Advanced Industrial Science and Technology  
National Metrology Institute of Japan  
Reference Material Certificate  
NMIJ CRM 6013-a  
No. +++  
L-Isoleucine

This certified reference material (CRM) was produced in accordance with the NMIJ’s management system and in compliance with JIS Q 0034 (ISO GUIDE 34). 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 validating analytical methods and instruments.

Certified Value
The certified value for the purity (in mass fraction) of L-isoleucine 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%.

<table>
<thead>
<tr>
<th>CAS No</th>
<th>Certified value, Mass fraction (kg/kg)</th>
<th>Expanded uncertainty, Mass fraction (kg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>L-Isoleucine ((2S,3S)-2-amino-3-methyl pentanoic acid)</td>
<td>73-32-5</td>
<td>0.997</td>
</tr>
</tbody>
</table>

The certified value and expanded uncertainty of isoleucine without enantiomeric separation is given in the table below, where the mass fraction of D-isoleucine is negligible.

<table>
<thead>
<tr>
<th>Certified value, Mass fraction (kg/kg)</th>
<th>Expanded uncertainty, Mass fraction (kg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Isoleucine (without enantiomeric separation)</td>
<td>0.997</td>
</tr>
</tbody>
</table>

Analysis
The certified value is based on the results of acidimetric titration, nitrogen determination by the Kjeldahl method and impurity determination by high performance liquid chromatography (HPLC). Impurities of amino acids were quantified by liquid chromatography mass spectrometry (LC/MS) and by LC with fluorescence detection after derivatization using o-phthalaldehyde (OPA). D-isoleucine 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 NIST SRM 351a (sodium carbonate) as the primary standards and by the impurity determination using the HPLC calibrated with purity-evaluated amino acids. It is traceable to the International System of Units (SI).

Expiration of Certification
This certificate is valid until March 31, 2020, provided that the material remains unopened and stored in accordance with the instructions given in this certificate.
Sample Form
This CRM is in the form of a white powder. A small amount (0.5 g) of this material was bottled in a glass vial and kept in an aluminum-laminated bag.

Homogeneity
The homogeneity of this CRM was determined by measured by acidimetric titration for 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 kept in a clean desiccator at room temperature (15 °C to 25 °C) 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 as soon as possible after opening. The CRM is for laboratory use only and not for in vivo use.

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

Preparation Method
Preparation of the material was performed by Wako Pure Chemical Industries, Ltd. Highly purified L-isoleucine provided by AJINOMOTO Co., Inc. was bottled into vials under argon atmosphere and each vial was sealed in an aluminum-laminated bag.

Information
The following values at the time of the certification are not certified but are provided as information. Impurities determined by HPLC were listed below. Moisture was determined by Karl Fischer titration to be 0.01 g/kg. The mass fraction of D-isoleucine determined by LC/MS using a chiral resolution column was less than 0.6 mg/kg.

<table>
<thead>
<tr>
<th></th>
<th>Reference value (g/kg)</th>
<th>Expanded uncertainty (g/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>α-aminobutyric acid</td>
<td>0.58</td>
<td>0.11</td>
</tr>
<tr>
<td>leucine</td>
<td>0.84</td>
<td>0.20</td>
</tr>
<tr>
<td>norvaline</td>
<td>0.39</td>
<td>0.09</td>
</tr>
<tr>
<td>tyrosine</td>
<td>0.13</td>
<td>0.03</td>
</tr>
<tr>
<td>valine</td>
<td>0.71</td>
<td>0.04</td>
</tr>
</tbody>
</table>

NMIJ Analysts
The technical manager for this CRM is A. Takatsu and production manager is M. Kato. The analysts are M. Kato, H. Kato, S. Eyama, M. Goto, and M. Yoshioka.

Technical Information
Customer registration on the NMIJ Website (given below) will facilitate notification of any revision of the information given above. 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, 2015

Ryoji Chubachi
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://www.nmij.jp/english/service/C/

Revision history
April 1, 2015: “Metrology Management Center” was renamed to “Center for Quality Management of Metrology.”