National Institute of Advanced Industrial Science and Technology

National Metrology Institute of Japan

Reference Material Certificate
NMIJ CRM 6026-a
No. +++
L-Glutamic acid

This certified reference material (CRM) was produced in accordance with the NMIJ’s management system and in compliance with ISO GUIDE 34:2009 and ISO/IEC 17025:2005. This CRM is primarily intended for use in calibrating the analytical instruments and reagents in amino acid analysis. It is also intended for controlling the precision of analysis, and for confirming the validity of analytical methods and instruments.

Certified Values
The certified value for the purity (in mass fraction) of L-glutamic acid 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-Glutamic acid</td>
<td>56-86-0</td>
<td>0.998</td>
</tr>
</tbody>
</table>

The certified value and expanded uncertainty for glutamic acid without enantiomeric separation is given in the table below, where the mass fraction of D-glutamic acid 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%.

<table>
<thead>
<tr>
<th>Certified value, Mass fraction (kg/kg)</th>
<th>Expanded uncertainty, Mass fraction (kg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glutamic acid (without enantiomeric separation)</td>
<td>0.998</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 LC with fluorescence detection after derivatization using o-phthalaldehyde (OPA) and LC with a charged aerosol detector (LC-CAD). D-glutamic acid was determined by LC/MS using a chiral resolution column. The certified value is the weighted mean of purities determined by acidimetric titration and nitrogen determination. The uncertainty was the combination of standard uncertainties due to purity determinations, differences between the methods, stability, and homogeneity.

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 until March 31, 2022, provided that the material 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 ca. 500 mg in net volume is kept in a brown 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 200 vials. The homogeneity is reflected in the uncertainty of the certified value.

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
From homogeneity, a minimum sample mass of 40 mg should be used. This 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
Wear protective equipment such as safety glasses, safety mask, and safety gloves in handling. 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-glutamic acid provided by AJINOMOTO Co., Inc. was bottled into vials under argon atmosphere. Each vial was sealed in an aluminum-laminated bag.

Technical Information
Impurities contained over 0.1 g/kg at the time of certification were aspartic acid and α-aminoadipic acid and their mass fractions were 0.80 g/kg and 0.13 g/kg, respectively.

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

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, 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.”
Jan. 28, 2019: The description on “Mutual Recognition Arrangement under Meter Convention” was added.