National Institute of Advanced Industrial Science and Technology
National Metrology Institute of Japan
Reference Material Certificate
NMIJ CRM 6025-a
No. +++
L-Cystine

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. It is primarily intended for use in calibrating analytical instruments and the preparation of standard solutions 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 Value
The certified values of purity (in mass fraction) are 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%.

Purity (in mass fraction) of L-cystine is given as follows.

<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>56-89-3</td>
<td>0.998</td>
<td>0.003</td>
</tr>
</tbody>
</table>

Purity (in mass fraction) of cystine including D- and meso- isomers is given in the table below.

<table>
<thead>
<tr>
<th>Certified value, Mass fraction (kg/kg)</th>
<th>Expanded uncertainty, Mass fraction (kg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.998</td>
<td>0.003</td>
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</table>

Analysis
These certified values are 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 identified and quantified by HPLC with visible detection after derivatization using ninhydrin and by liquid chromatography/mass spectrometry (LC/MS). The certified value is the weighted mean of purities, as determined by acidimetric titration and nitrogen determination. The uncertainty is the combination of standard uncertainties due to purity determinations, the differences between the methods and homogeneity.

Metrological Traceability
The certified value was determined using titrimetry as the primary method of measurement, with NMIJ CRM 3001-a (potassium hydrogen phthalate) and NMIJ CRM 3005-a (sodium carbonate) as the primary standards, and by impurity determination using a HPLC calibrated with purity-evaluated amino acids. It is traceable to the International System of Units (SI).
Expiration of Certification
This certificate is valid for one year from the date of shipment, 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. 500 mg of the material is bottled in a brown glass vial and kept in an aluminum-laminated bag.

Homogeneity
The homogeneity of this CRM was evaluated by acidimetric titration, analyzing 12 vials randomly selected from a group of 200 vials. The uncertainty of homogeneity is reflected in the uncertainty of the certified value.

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

Instructions for Use
From the homogeneity, a minimum sample mass of 30 mg should be used. This CRM is for laboratory use only and not for in vivo use. The CRM should be used promptly once a bottle is opened. In preparing standard solutions, the stability of the cystine standard solution should be considered, as cystine in aqueous solution is known to be gradually decomposed into cysteine.

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-cystine provided by Wako Pure Chemical Industries, Ltd. was bottled into vials under argon atmosphere, and each vial was then sealed in an aluminum-laminated bag.

Information
At the time of the certification, the mass fraction of meso-cystine was approximately 0.2 g/kg, and the mass fraction of cysteine was under 0.1 g/kg.

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.

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:
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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/

<table>
<thead>
<tr>
<th>Revision history</th>
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<tbody>
<tr>
<td>April 1, 2015:</td>
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<tr>
<td>“Metrology Management Center” was renamed to “Center for Quality Management of Metrology.”</td>
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<tr>
<td>Nov 20, 2017:</td>
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<tr>
<td>The description in “Expiration of Certification” was changed to “one year from the date of shipment.”</td>
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