National Institute of Advanced Industrial Science and Technology

National Metrology Institute of Japan

Reference Material Certificate
NMIJ CRM 6206-a
No. +++
Okadaic Acid Standard Solution

This certified reference material (CRM), okadaic acid solution (dissolved in methanol containing 0.5% (volume fraction) ethanol), 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 intended for use in the preparation of standard solutions for the determination of okadaic acid in diarrhetic shellfish toxin testing.

Certified Value
The certified value for the mass concentration of okadaic acid at 25 °C 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>Compound</th>
<th>CAS No.</th>
<th>Certified value, Mass concentration (µg/mL)</th>
<th>Expanded uncertainty Mass concentration (µg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Okadaic Acid</td>
<td>78111-17-8</td>
<td>0.909</td>
<td>0.073</td>
</tr>
</tbody>
</table>

Analysis
This CRM was prepared by the gravimetric blending of the solvent (0.5% (volume fraction) ethanol in methanol) and okadaic acid solution, in which the mass fraction of okadaic acid was determined by a quantitative NMR method. The mass concentration is obtained from the mass fraction of okadaic acid and the density of the diluted solution. The standard uncertainties due to NMR measurement, dilution, homogeneity, stability and the temperature-related variation in density are combined. The uncertainty of the quantitative NMR method was estimated from a collaborative study, and is included in the uncertainty of NMR measurement.

Metrological Traceability
In the quantitative NMR method, a 1,4-bis(trimethylsilyl)benzene solution in methanol, calibrated using 3,5-bis(trifluoromethyl)benzoic acid (certified reference material (NMIJ CRM 4601-a)), was used as an internal standard. A Japan Calibration Service System (JCSS) calibrated balance was used for gravimetric dilution of the okadaic acid solution. The certified value is traceable to the International System of Units (SI).

Indicative Values
The indicative values for mass fraction and amount of substance concentration of okadaic acid at 25 °C are given in the table below. The uncertainty of the indicative 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>Compound</th>
<th>Indicative value</th>
<th>Expanded uncertainty</th>
</tr>
</thead>
<tbody>
<tr>
<td>Okadaic Acid</td>
<td>Mass fraction 1.155 µg/g</td>
<td>0.093 µg/g</td>
</tr>
<tr>
<td></td>
<td>Amount of substance concentration 1.129 µmol/L</td>
<td>0.091 µmol/L</td>
</tr>
</tbody>
</table>
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 colorless clear liquid. This CRM of ca. 1 mL in net volume is kept in an amber ampule.

Homogeneity
The homogeneity of this CRM was determined by analyzing 10 ampules selected from among 522 ampules by stratified random sampling in order of subdivision. The homogeneity of this CRM was evaluated from the peak area of okadaic acid by HPLC-UV. The homogeneity is reflected in the uncertainty of the certified value.

Instructions for Storage
This CRM should be stored below –20 °C and shielded from light.

Instructions for Use
This CRM should be used between 20 °C and 30 °C. The ampule of this CRM should be warmed to between 20 °C and 30 °C before opening and used promptly once the ampule is opened. Evaporation of methanol should be avoided. This CRM is for laboratory use only.

Precautions for Handling
Wear a mask, gloves, and other protective equipment during handling. Be careful to avoid injury when opening the glass ampoule. Follow local rules or regulations for the disposal of this CRM or diluted solutions. Refer to the safety data sheet (SDS) on this CRM before use.

Preparation
Okadaic acid was produced by a large culture of the toxic dinoflagellate Prorocentrum lima, and isolated by liquid-liquid partitioning and several column chromatography steps at National Research Institute of Fisheries Science (NRIFS). The CRM solution was prepared by dissolving purified okadaic acid in methanol containing ethanol and dispensed into amber glass ampoules at NMIJ.

Technical Information
The density of this CRM is 0.7871 g/cm³ at 25 °C and it will change by approximately 0.6 % for a 5 °C change in temperature. The molar mass of okadaic acid is 805.00 g/mol. The structure of okadaic acid is shown below.

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

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.

Note
This CRM was developed in collaboration with National Research Institute of Fisheries Science (NRIFS), with the support of a grant from Cross-ministerial Strategic Innovation Promotion Program (SIP), “Technologies for creating next-generation agriculture, forestry and fisheries” (funding agency: Bio-oriented Technology Research Advancement Institution).
Production of this CRM was based on the notable research achievements made by Prof. Takeshi Yasumoto in the discovery of diarrhetic shellfish toxins and the production of their standards.

March 9, 2016
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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