

National Institute of Advanced Industrial Science and Technology

## National Metrology Institute of Japan



## Reference Material Certificate

NMIJ CRM 7521-a

No. +++



## Diarrhetic Shellfish Toxins in Scallop Edible Parts

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. This CRM is intended for use in the accuracy control of analyses and the validation of analytical techniques for the determination of diarrhetic shellfish toxins in scallop edible parts and similar materials in accordance with Notice issued by the Ministry of Health, Labour and Welfare, Japan (Shoku-An-Ki 030603 and Shoku-An-Kan 030601, 2015).

**Certified Values**

The certified value of okadaic acid and dinophysistoxin-1 (DTX1) of this CRM is given in the table below. The uncertainty of the certified value is the expanded uncertainty obtained by multiplying the combined standard uncertainty by a coverage factor ( $k$ ) of 2, and it is the half-width of an interval estimated to have a level of confidence of approximately 95 %.

Substance	CAS No.	Certified value Mass fraction (mg/kg)	Expanded uncertainty Mass fraction (mg/kg)
Okadaic acid	78111-17-8	0.056	0.019
DTX1	81720-10-7	0.057	0.019

**Analysis**

The certified values of this CRM were obtained based on the results of the analyses conducted by the eight participants (listed in Note) of the interlaboratory certification study. Each participant employed an analytical method, following the performance criteria stipulated in Appendix of Notice issued by the Ministry of Health, Labour and Welfare, Japan (Shoku-An-Ki 030603 and Shoku-An-Kan 030601, 2015), and quantified okadaic acid and DTX1 by using a standard addition method approach.

The outline of the analytical method exemplified in Annex 2 of this Notice is as follows:

[Preparation] extraction using methanol and 90% methanol, hydrolysis by adding sodium hydroxide, washing with hexane, and clean-up by solid-phase extraction using an octadecylsilyl-silica gel (ODS) cartridge

[Liquid chromatography-tandem mass spectrometric conditions] separation column: ODS column; ionization method: electrospray ionization; ionization mode: negative; detection: selected reaction monitoring

**Metrological Traceability**

The certified values of this CRM are the concentrations of okadaic acid and DTX1 quantified by the analytical method described in Analysis. The standard solution used for the interlaboratory certification study was prepared gravimetrically from NMIJ CRM 6206-a (Okadaic Acid Standard Solution) and NMIJ CRM 6207-a (Dinophysistoxin-1 (DTX1) Standard Solution).

**Expiration of Certification**

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

**Description of the material**

The raw materials of this CRM were obtained from shucked non-toxic raw scallop and toxic midgut gland. These raw materials were processed to be homogenized. After the spiking of okadaic acid and dinophysistoxin-2 (DTX2), they were mixed, bottled,

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and sterilized by the  $\gamma$ -ray irradiation (20 kGy). This CRM is in the form of ocher paste at room temperature, and 10 g of this CRM in net volume is packaged in a plastic vial.

#### Instructions for Storage

This CRM should be stored at a temperature of  $-30\text{ }^{\circ}\text{C}$  to  $-20\text{ }^{\circ}\text{C}$  and protected from sunlight.

#### Instructions for Use

A vial of this CRM should be allowed to warm to room temperature of  $15\text{ }^{\circ}\text{C}$  to  $25\text{ }^{\circ}\text{C}$  for 8 to 15 hours before opening to thaw the CRM completely. This CRM should be used promptly once a vial is opened. This CRM should be well mixed for 5 min, by using a spatula for instance, before it is sampled. Considering the homogeneity, a minimum sample size of more than 0.5 g should be used to ensure valid results.

#### Precautions for Handling

A protective mask, gloves, and other protective gears should be used for safety when this CRM is used. Refer to the safety data sheet (SDS) on this CRM before use. This CRM must be disposed of in accordance with all relevant laws regarding waste handling and management.

#### Preparation

The raw materials of this CRM were obtained from shucked non-toxic raw scallop and toxic midgut gland. These raw materials were processed to be homogenized. After the spiking of okadaic acid and DTX2, they were mixed, bottled, and sterilized by the  $\gamma$ -ray irradiation (20 kGy). The okadaic acid used was prepared by National Research Institute of Fisheries Science (NRIFS), using a large culture of the toxic dinoflagellate *Prorocentrum lima*. The DTX2 (CRM-DTX2-b) used was purchased from NRC Canada. Part of the preparation of the CRM was performed by the General Environmental Technos Co., Ltd under the contract with NMIJ.

#### Technical Information

At the time of certification, the mass fraction of DTX2 in this CRM was approximately 0.011 mg/kg.

#### NMIJ Analysts

The technical manager for this CRM is KATO M., the production manager is KAWAGUCHI M., and the analysts are KAWAGUCHI M., EYAMA S., MIYAMOTO A., INAGAKI S., and YAMAZAKI T.

#### Information

If substantive technical changes occur that affect the certification before the expiration of this certificate, NMIJ will notify the registered customers. 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 provided by Government of Japan and grants from the Project of the NARO Bio-oriented Technology Research Advancement Institution (the special scheme project on vitalizing management entities of agriculture, forestry and fisheries).

The following institutes participated in the interlaboratory certification study: National Metrology Institute of Japan (NMIJ), National Research Institute of Fisheries Science (NRIFS), Food and Agricultural Materials Inspection Center (FAMIC), Kanagawa Prefectural Institute of Public Health, Japan Food Research Laboratories (JFRL), Japan Foods Inspection Corporation (JFIC), Aomori Pharmaceutical Association Food and Water Inspection Center, and Shimadzu Corporation (listed in random order).

The scallops used as the raw material of this CRM were kindly provided by Aomori Prefecture Scallop Marketing Promotion

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Association Japan.

April 1, 2020

ISHIMURA Kazuhiko  
President

National Institute of Advanced Industrial Science and Technology

If you have any questions about this CRM, please contact:  
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Revision history

June 6, 2019: Expanded uncertainties of “Certified value” of Okadaic acid and DTX1 were changed.