

National Institute of Advanced Industrial Science and Technology

## National Metrology Institute of Japan



## Reference Material Certificate

NMIJ CRM 6207-a  
No. +++

## Dinophysistoxin-1 (DTX1) Standard Solution

This certified reference material (CRM), DTX1 solution (dissolved in methanol containing 0.5 % (volume fraction) ethanol), 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 preparation of standard solutions for the determination of DTX1 in diarrhetic shellfish toxin testing.

**Certified Value**

The certified value for the mass concentration of DTX1 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 %.

Compound	CAS No.	Certified value, Mass concentration ( $\mu\text{g/mL}$ )	Expanded uncertainty Mass concentration ( $\mu\text{g/mL}$ )
DTX1	81720-10-7	1.079	0.078

**Analysis**

This CRM was prepared by the gravimetric blending of the solvent (0.5 % ethanol (volume fraction) in methanol) and DTX1 solution, in which the mass fraction of DTX1 was determined by a quantitative NMR method. The mass concentration is obtained from the mass fraction of DTX1 and the density of the diluted solution. The standard uncertainties due to the 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 the 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 DTX1 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 DTX1 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 %.

Compound		Indicative value	Expanded uncertainty
DTX1	Mass fraction	1.370 $\mu\text{g/g}$	0.099 $\mu\text{g/g}$
	Amount of substance concentration	1.317 $\mu\text{mol/L}$	0.095 $\mu\text{mol/L}$

**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 567 ampules by stratified random sampling in order of subdivision. The homogeneity of this CRM was evaluated from the peak area of DTX1 by HPLC-UV. The homogeneity is reflected in the uncertainty of the certified value.

### Instructions for Storage

This CRM should be stored below  $-20\text{ }^{\circ}\text{C}$  and shielded from light.

### Instructions for Use

This CRM should be used between  $20\text{ }^{\circ}\text{C}$  and  $30\text{ }^{\circ}\text{C}$ . This CRM should be warmed to between  $20\text{ }^{\circ}\text{C}$  and  $30\text{ }^{\circ}\text{C}$  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

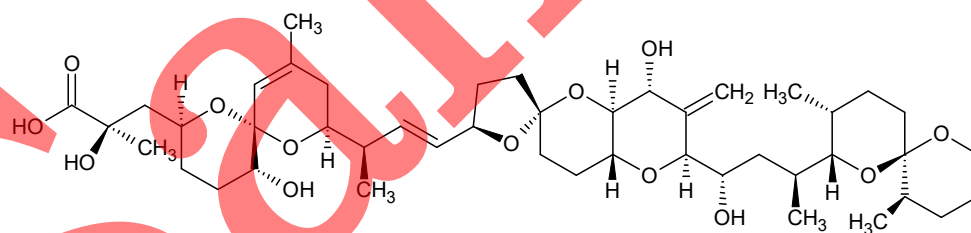
A mask, gloves, and other protective equipment should be worn during handling. Refer to the safety data sheet (SDS) on this CRM before use. Be careful to avoid injury when opening the glass ampoule. Follow local rules or regulations for the disposal of this CRM or diluted solutions.

### Preparation

DTX1 was produced by a large culture of the toxic dinoflagellate *Prorocentrum lima* and isolated by liquid-liquid partitioning and several columns chromatography steps at National Research Institute of Fisheries Science (NRIFS). The CRM solution was prepared by dissolving purified DTX1 in methanol containing ethanol and dispensed into amber glass ampoules at NMIJ.

### Technical Information

The density of this CRM is  $0.7872\text{ g/cm}^3$  at  $25\text{ }^{\circ}\text{C}$  and it will change by approximately 0.6 % for a  $5\text{ }^{\circ}\text{C}$  change in temperature. The molar mass of DTX1 is  $819.0275\text{ g/mol}$ . The structure of DTX1 is shown below.



### NMIJ Analysts

The technical and production managers for this CRM are TAKATSU A. and the analysts are KAWAGUCHI M., YAMAZAKI T. and EYAMA S.

### 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. YASUMOTO Takeshi in the discovery of diarrhetic shellfish toxins and the production of their standards.

April 1, 2020

ISHIMURA Kazuhiko  
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://unit.aist.go.jp/nmij/english/refmate/>

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