

National Institute of Advanced Industrial Science and Technology

## National Metrology Institute of Japan



## Reference Material Certificate

NMIJ CRM 6001-a

No. +++

Cholesterol



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 calibration of analytical instruments, quality control of analytical instruments, and validation of analytical techniques and instruments.

**Certified Value**

The certified value of purity (mass fraction) of this CRM 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 %.

	CAS No.	Certified value, Mass fraction (kg/kg)	Expanded uncertainty, Mass fraction (kg/kg)
Cholesterol	57-88-5	0.999	0.001

**Analysis**

The certified value for this CRM is the purity in mass fraction, converted from the purity in the amount-of-substance fraction obtained by the freezing point depression method using the average molecular weight of impurities. In the freezing point depression method, the purity in the amount-of-substance fraction was obtained by the stepwise scan method carried out using a differential scanning calorimeter (DSC). The average molecular weights of impurities were estimated using their concentrations determined by gas chromatography/mass spectrometry (GC/MS), high-performance liquid chromatography/ultraviolet absorption detection (HPLC/UV), and Karl-Fischer titration. Expanded uncertainty was calculated from combined standard uncertainty due to uncertainties in purity determination and homogeneity, and a coverage factor ( $k$ ) of 2.

**Metrological Traceability**

The certified value was determined by the freezing point depression method carried out using a DSC, the temperature and enthalpy of which were calibrated in accordance with NIST SRM 2225 (Mercury) and NIST SRM 2232 (Indium), respectively. The certified value was traceable to the International System of Units (SI).

**Mutual Recognition Arrangement under Metre Convention**

This certificate 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 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 at room temperature. This CRM of *ca.* 1 g in net volume is kept in an amber glass vial with argon gas. The glass vial is sealed in an aluminum-laminated plastic bag.

**Homogeneity**

Ten vials were sampled from 350 vials at almost equal intervals in the order of subdivision for the homogeneity test by HPLC and DSC. The area percentage of cholesterol (determined by HPLC) and total concentration of impurities (determined by DSC) were measured and uncertainties in homogeneity were estimated by an analysis of variance of the results. The estimated uncertainties were taken into account for determining the uncertainty in the certified value.

**Precautions for Storage**

This CRM should be stored at a temperature between 0 °C and 6 °C in a dark place.

**Instructions for Use**

This CRM is for laboratory use only and not for *in vivo* use. The vial of this CRM should be allowed to warm to room temperature before opening. This CRM is hygroscopic and should be used promptly once the vial is opened.

**Precautions for Handling**

Protective equipment such as safety glasses, safety mask, and safety gloves should be worn while handling this CRM. Refer to the safety data sheet (SDS) on this material before use.

**Preparation**

This CRM was purified and subdivided by Kishida Chemical Co., Ltd. Companion steroids were removed by treating the CRM with bromine and dehalogenation with zinc. Cholesterol was recrystallized from ethanol–acetone. The material was bottled in amber glass vials and sealed in aluminum laminated bags under an argon atmosphere.

**Technical Information**

At the time of certification on March 2005, the concentrations of desmosterol and campesterol determined by GC/MS and HPLC were 0.5 mg/g and 0.1 mg/g, respectively. The concentration of  $\beta$ -cholestanol determined by GC/MS was 0.2 mg/g. The concentrations of  $\beta$ -sitosterol, 7-dehydrocholesterol, and lathosterol determined by GC/MS were less than 0.1 mg/g. No impurities were detected by nuclear magnetic resonance and infrared spectroscopy. The melting point measured by DSC was in the range from 149.0 °C to 149.2 °C. The concentration of Br determined by X-ray fluorescence analysis was 13  $\mu$ g/g.

**NMIJ Analysts**

The technical managers for this CRM are KATO K. and TAKATSU A. The production manager is SHIMIZU Y., and the analysts are SHIMIZU Y., TAKATSU A., ISHIKAWA K., BAO X., IWASAWA R., OHTE Y. and SAEKI M.

**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, 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

December 20, 2013: The expiration date of this certificate was changed to March 31, 2021, from March 31, 2015.

Format of the table of certified value was revised.

Descriptions on Metrological Traceability, Mutual Recognition Arrangement under Metre Convention, and Precautions for Handling were added.

April 1, 2015: “Metrology Management Center” was renamed to “Center for Quality Management of Metrology.”

July 18, 2019: The description in “Expiration of Certification” was changed to “one year from the date of shipment.”