National Institute of Advanced Industrial Science and Technology

National Metrology Institute of Japan

Reference Material Certificate

NMIJ CRM 6901-b
No. +++

C-peptide

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. This CRM is the lyophilized synthetic peptide having a human C-peptide sequence. This CRM is primarily intended for use in calibrating and controlling the precision of instruments for the determination of C-peptide, and for value-assignment of calibrator. It can also be used to control the precision and confirm the validity of analytical methods in amino acid analysis. When the material is used in a particular assay, the commutability should be verified.

Certified Values

When the lyophilized material is reconstituted at 20 °C according to the specified procedure (see "Instructions for Use"), the solution of total C-peptide (mixture of C-peptide, deamidated C-peptide, and pyroglutamylated C-peptide) in 10 mmol/L phosphate buffer (pH 6.6) is obtained.

The certified values for the mass concentration of C-peptide and total C-peptide at 20 °C are given in the tables 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 concentration (mg/L)	Expanded uncertainty Mass concentration (mg/L)
C-peptide	33017-11-1	100	5

	Certified value Mass concentration (mg/L)	Expanded uncertainty Mass concentration (mg/L)
Total C-peptide (Mixture of C-peptide, deamidated C-peptide, and pyroglutamylated C-peptide)	102	5

Analysis

The certified values were determined as follows: Amino acid analysis employing an isotope-dilution mass spectrometry was performed for the solution reconstituted with 1.00 g of double-distilled water. The content of C-peptide in total C-peptide was determined by a high performance liquid chromatography. The result of amino acid analyses were converted into the mass concentration by the density and molecular weight to give the certified values.

Amino acid analyses were conducted by the following two different methods.

1) Liquid-phase hydrolysis followed by reversed phase chromatography/mass spectrometry utilizing the pre-column derivatization method:

The hydrolysis was performed by microwave-assisted liquid-phase hydrolysis with hydrochloric acid at 165 °C for 3 h. The hydrolyzed amino acids, glycine, glutamic acid, proline, alanine, valine, and leucine were quantified using *N*-butylnicotinic acid *N*-hydroxysuccinimide ester iodide as the derivatization reagent.

2) Gas-phase hydrolysis followed by hydrophilic chromatography/mass spectrometry method:

Gas-phase hydrolysis was performed at 130 °C for 48 h. The hydrolyzed amino acids, proline, alanine, valine, and leucine were quantified.

The concentration of peptide was calculated based on the numbers of constituted amino acids of C-peptide. The quantitative results were obtained by calculating the weighted average of two amino acid analyses. This value represents the concentration of total C-peptide (total concentration of C-peptide, deamidated C-peptide, and pyroglutamylated C-peptide). The concentration of C-peptide was calculated by the ratio between C-peptide to total C-peptide determined by chromatography.

Metrological Traceability

Each certified value is traceable to the International System of Units (SI) via amino acid analysis based on the primary method, isotope-dilution mass spectrometry calibrated with L-alanine (NMIJ CRM 6011-a), L-leucine (NMIJ CRM 6012-a), L-valine (NMIJ CRM 6015-a), L-proline (NMIJ CRM 6016-a), glycine (NMIJ CRM 6022-a), and L-glutamic acid (NMIJ CRM 6026-a).

Expiration of Certification

This certificate is valid for one year from the date of shipment, provided that the material is stored in accordance with the instructions given in this certificate.

Sample Form

This CRM is in the form of a white lyophilized powder. This CRM of ca. 100 µg in net volume is kept in a glass vial. Each sample consists of lyophilized C-peptide and sodium phosphate.

Homogeneity

The homogeneity of this CRM was verified by measuring C-peptide and total C-peptide in 10 vials taken by stratified random sampling over 120 of the whole batch. The measurements were performed using reversed-phase chromatography using benzoic acid as an internal standard using 0.1 g of this material. ANOVA statistics were used to calculate the between-bottle standard deviation. The homogeneity is reflected in the uncertainty of the certified value.

Stability

The stability was confirmed, and reflected in the uncertainty of the certified values.

Instructions for Storage

This CRM should be stored in a freezer (lower than –20 °C) after receiving.

Instructions for Use

CAUTION: Do not open the rubber septum prior to reconstituting the content. The entire content of the vial must be reconstituted.

This CRM must be reconstituted according to the following procedure.

1. Take out the aluminum bag containing the vial from the freezer and allow the bag to stand at room temperature for 30 min.
2. Take out the vial from the aluminum bag and ensure the lyophilized pellet is at the bottom of the vial. If the lyophilized pellet is attached to the vial wall, tap the bottom of the vial gently on the table to move the pellet to the bottom of the vial.
3. Carefully remove the aluminum cap from the vial. At this time, do not open the rubber septum.
4. Weigh the vial with the rubber septum, using a balance having less than 0.1 mg of the verification scale interval.
5. Inject 1.00 g of water through the rubber septum using a syringe*. Make sure that the difference of the weights between the vial with water and that without water measured at step 4 is within 0.99 g to 1.01 g.
6. Shake the vial lightly to dissolve the material completely and allow the vial to stand for 10 min.
7. The reconstituted material should be used within 24 h.

*The use of a microsyringe having at least 1 % of accuracy is recommended. Prior to its use for reconstitution, the microsyringe should be checked by using a balance. To inject precisely 1.00 g of water for the reconstitution, water may be ejected several times prior to reconstitution, checking the amount of water ejected by the balance.

Instructions for Use

C-peptide may tend to be adsorbed on the surface of labware. It is recommended that low-adsorption material and a buffer containing a carrier protein be used to handle the reconstituted material. The certified value as total C-peptide should be used for the analyses, e.g., amino acid analysis and the immunochemical method, which cannot differentiate between C-peptide and the modified form of C-peptide, such as deamidated and pyroglutamylated C-peptide.

Precautions for Handling

This CRM is for laboratory use only and not for *in vivo* use. Refer to the safety data sheet (SDS) on this CRM before use.

Preparation

C-peptide was synthesized and purified by Peptide Institute Inc., Osaka, Japan. C-peptide was dissolved in phosphate buffer and aliquoted in a glass vial at NMIJ. After the material was lyophilized, the vials were sealed with rubber septum in nitrogen atmosphere.

Technical Information

The density of the reconstituted solution is 0.9993 g/mL at 20 °C.

Ten mmol/L of phosphate buffer contains 6.2 mmol/L of NaH_2PO_4 and 3.8 mmol/L of Na_2HPO_4.

This material contains (7.0 ± 0.1) mg/L of trifluoroacetate, after reconstitution according to “Instruction for Use.” The numeric value after the symbol ± indicates the standard deviation of measurement.

The amino acid sequence of human C-peptide is as follows: EAEDLQVGQVELGGGPGAGSLQPLALEGSLQ

Molecular weight of C-peptide is 3020.26, and its monoisotopic mass is 3018.52.

NMIJ Analysts

The technical manager for this CRM is A. Takatsu and the production manager is T. Kinumi. The analysts are T. Kinumi, M. Kato, T. Yamazaki, and R. Mizuno.

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, 2015

Ryoji Chubachi
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 (NMIJ),
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/

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