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Shipping date : 20**.**

National Institute of Advanced Industrial Science and Technology

National Metrology Institute of Japan

Reference Material Certificate

NMIJ CRM 6901-a
No. +++

C-Peptide

National Institute of Advanced Industrial Science and Technology
AIST

This certified reference material (CRM) was produced based on NMIJ's quality system in compliance with ISO GUIDE 34:2000 and ISO/IEC 17025:2005, and was the lyophilized synthetic peptide having human C-peptide sequence. The material is primarily intended to be used to calibrate and control the precision of instruments for the determination of C-peptide, and for value-assignment of calibrator. It can also be used for controlling the precision and confirming 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 according to the specified procedure (see "Instruction 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 following tables. The expanded uncertainty was determined using coverage factor k =2, corresponding to an estimated confidence interval of approximately 95 %.

	CAS No.	Certified value, Mass concentration (mg/L)	Expanded uncertainty, Mass concentration (mg/L)
C-peptide	33017-11-1	80.7	5.0

The certified value is given for the reconstituted solution according to "Instruction for Use".

	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)	81.7	5.1

The certified value is given for the reconstituted solution according to "Instruction for Use".

Analytical Methods

The certified values were determined as follows: Amino acid analysis employing 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 with high performance liquid chromatography. The obtained amount of substance content was converted into the mass concentration by the density and molecular weight to give the certified values.

Amino acid analyses were conduced by the following two different methods for the hydrolyzed amino acids generated by gas phase hydrolysis at 130 °C for 48 hours.

1) Reversed phase chromatography/mass spectrometry utilizing pre-column derivatization method:

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.

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2) Hydrophilic chromatography/mass spectrometry method:

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. 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 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), and purity-defined glycine and L-glutamic acid.

Stability

The stability is confirmed by stability study during a period of an approximately half a year. This CRM is kept at -80 °C in NMIJ, and the stability has been monitored every six months.

Expiration of Certification

This certificate is valid for 3 months after purchase under the specified storage condition below.

Sample Form

Each sample consists of white lyophilized C-peptide (approximately 80 μg) and sodium phosphate in 10 mL glass vial.

Homogeneity

The homogeneity of the CRM was verified by measuring C-peptide and total C-peptide in 9 vials taken randomly over 109 of the whole batch. The measurements were performed reversed phase chromatography using benzoic acid as an internal standard. ANOVA statistics were used to calculate the between bottle standard deviation, which was included in the uncertainties of the certified values.

Storage

This CRM should be kept in a freezer (less than -20 °C) after purchase.

Instruction 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 has to be reconstituted according to the following procedure.

1. Remove the vial containing in the aluminum bag from the freezer, and place the bag at room temperature for 1 hour.
2. Remove the vial from the aluminum bag, and ensure the lyophilized pellet is at the bottom of the vial. If the lyophilized pellet is come off from the vial wall, tap the bottom of the vial gently on the table.
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 the balance having less than 0.1 mg of the reciprocal sensibility.
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 without water measured at 4 is within 0.99 g - 1.01 g.
6. Shake lightly to dissolve the material completely, and leave to stand for 20 min.
7. The reconstituted material should be used within 12 hours.

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

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Precautions for Use

C-peptide may tend to be adsorbed on the surface of labware. It is recommended that the use of low adsorption material and buffer containing carrier protein for the dilution to handle the reconstituted material.

The certified value as total C-peptide should be used for the analyses e.g. amino acid analysis and immunochemical method which can not differentiate between C-peptide and the modified form of C-peptide such as deamidated and pyroglutamylated C-peptide.

This CRM is for laboratory use only and not for *in vivo* use.

Preparation Method

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

Technical Information

Reconstituted solution contains (0.46 ± 0.08) mg/L of deamidated C-peptide and (0.57± 0.04) mg/L of pyroglutamylated C-peptide. The numeric value after the symbol ± indicates expanded uncertainty ($k = 2$).

The density of the reconstituted solution is 0.9994 g/mL at 20 °C.

Ten mmol/L of phosphate buffer contains 6.24 mmol/L of NaH_2PO_4 and 3.76 mmmol/L of Na_2HPO_4.

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 production manager is T. Kinumi. The analysts are T. Kinumi, M. Kato, T. Yamazaki, M. Goto, and S. Eyama.

Information

Customer registration on the NMIJ WEB site shown below will facilitate notification of above revision. Technical report about this CRM can be also obtained from the contact shown below.

Reproduction of Certificate

In reproducing this certificate, it should be clearly indicated that the document is a copy.

April 13, 2011

Tamotsu Nomakuchi
President
National Institute of Advanced Industrial Science and Technology

If you have any questions about this CRM, please contact
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