National Institute of Advanced Industrial Science and Technology

National Metrology Institute of Japan

Reference Material Certificate

NMIJ CRM 6901-c
No. +++

C-peptide

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 the lyophilized synthetic peptide having a human C-peptide sequence. This CRM is primarily intended for use in calibration of analytical instruments and in control of the accuracy and validation of analytical procedure, and for value-assignment of calibrator. It can also be used to control the precision and confirm the validity of analytical methods and analytical instruments in amino acid analysis.

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 concentrations of C-peptide and total C-peptide at 20 °C are given in the tables 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 concentration (mg/L)	Expanded uncertainty Mass concentration (mg/L)
C-peptide	33017-11-7	104	5

Substance	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)	105	5

Analysis

The certified values were determined as follows: The amino acid analyses employing an isotope-dilution mass spectrometry were performed for the solution reconstituted with 1.00 g of purified water. The content of C-peptide in total C-peptide was determined by a high performance liquid chromatography. The peptide impurities were assessed by a high-resolution mass spectrometry. The results of amino acid analyses above-mentioned were converted into the mass concentration by the density and molar mass to give the certified values.

Amino acid analyses were conducted by the following two different methods after spiking isotopically labeled amino acids.

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 as the derivatization reagent.

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

Gas-phase hydrolysis was performed at 130 °C for 24 h. The hydrolyzed amino acids, glycine, glutamic acid, proline, alanine, valine, and leucine were quantified.

The concentration of total C-peptide (total concentration of C-peptide, deamidated C-peptide, and pyroglutamylated C-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 the results by the two amino acid analyses. The concentration of C-peptide was calculated by the ratio of C-peptide to total C-peptide determined by high performance liquid 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 the certified reference materials, 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). The mechanical oscillator densitometer was calibrated with density standard liquid from Japan Calibration Service System.

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.

Description of the Material

This CRM is in the form of a white lyophilized powder. This CRM of ca. 0.1 mg in net weight is kept in a glass vial. Each sample consists of lyophilized C-peptide and sodium phosphate.

Instructions for Storage

This CRM should be kept 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 should be reconstituted.

This CRM should 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 more than 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 or rubber septum, 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 purified water through the rubber septum using a syringe*. Make sure that the difference of the weights between the vial with purified water and that vial 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 stored at less than 5 °C and used within 24 h.

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

Information for Use

C-peptide and the modified forms 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 forms of C-peptide, such as deamidated and pyroglutamylated C-peptide.

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

Commutability

When the material is used in a immunoassay, the commutability should be verified.

Precautions for Handling

Refer to the safety data sheet (SDS) on this CRM before use.

Preparation

The raw material of C-peptide was synthesized and purified by Peptide Institute Inc., Osaka, Japan. This material 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

1) The density of the reconstituted solution is 0.9993 g/ cm^3 at 20 °C. The density at 5 °C is 1.001 g/ cm^3.
2) Ten mmol/L of phosphate buffer in the reconstituted solution is equivalent to mixture of 6.2 mmol/L of NaH_2PO_4 and 3.8 mmol/L of Na_2HPO_4.
3) This material contains (6.6 ± 0.1) mg/L of trifluoroacetate ion, after reconstitution according to "Instruction for Use." The numeric value after the symbol ± indicates the standard deviation of measurement.
4) The amino acid sequence of human C-peptide is as follows: EAEDLQVGQVELGGGPGAGSLQPLALEGSLQ
5) The molar mass of C-peptide is 3020.26 g/mol
6) The monoisotopic mass of C-peptide obtained by high-resolution mass spectrometry is 3018.51 u.

NMIJ Analysts

The technical manager for this CRM is KATO M., the production manager is KINUMI T., and the analysts are KINUMI T., SAIKUSA K., MIZUNO R. and EYAMA S.

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.

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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