Date of Shipment: Xxxxx xx, 20xx

6210a00-230224-240530

National Institute of Advanced Industrial Science and Technology

National Metrology Institute of Japan

Reference Material Certificate

NMIJ CRM 6210-a

No. +++

Amyloid β

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 amyloid β (1-42) sequence (thereafter, amyloid β (1-42) is defined as amyloid β). This CRM is primarily intended for use in calibrating and controlling the accuracy of instruments for the determination of amyloid β, and for value-assignment of calibrator. It can also be used to control the precision and confirm the validity of analytical methods and 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 amyloid β (mixture of amyloid β, oxdated amyloid β, deamidated amyloid β, and isomerized amyloid β) in 0.1 % aqueous ammonia solution is obtained.

The certified values for the mass concentration of amyloid β and total amyloid β at 20 ˚C are given in the table below. The uncertainty of the certified value is the expanded uncertainty obtained by multiplying the combined standard uncertainty by a coverage factor (k) of 2.45, 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)
Amyloid β	107761-42-2	42.6	7.0

Substance	Certified value Mass concentration (mg/L)	Expanded uncertainty Mass concentration (mg/L)
Total amyloid β (Mixture of amyloid β, oxdated amyloid β, deamidated amyloid β, and isomerized amyloid β)	46	11

Analysis

The certified values of this CRM, expressed as mass concentration, were determined as follows: the results of amino acid analysis employing an isotope-dilution mass spectrometry were converted by the density, which measured by the vibration type density meter, and molar masses to give the certified values. The peptide impurities were identifed by high-resolution mass spectrometry. Amino acid analyses were performed 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, alanine, aspartic acid, glutamic acid, isoleucine, leucine, phenylalanine, and valine were quantified using *N*-butylnicotinic acid *N*-hydroxysuccinimide ester as the derivatization reagent.

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

Gas-phase hydrolysis was performed at 150 ˚C for 24 h. The hydrolyzed amino acids, glutamic acid, isoleucine, leucine, phenylalanine, and valine were quantified.
The peptide concentration was calculated based on the number of constituted amino acids in amyloid β. The quantitative results were obtained by calculating the weighted mean of the results by two amino acid analyses. This value is the concetration of total amyloid β (total concetration of amyloid β, oxdated amyloid β, deamidated amyloid β, and isomerized amyloid β). The concetration of amyloid β was determined by multiplying the concentration of total amyloid β by the ratio of amyloid β to total amyloid β determined by high performance liquid chromatography.

Metrological Traceability

The certified values of this CRM were determined by amino acid analyses employing isotope dilution-mass spectrometry calibrated with the certified reference materials, L-alanine (NMIJ CRM 6011-a), L-aspartic acid (NMIJ CRM 6027-a), L-glutamic acid (NMIJ CRM 6026-a), L-isoleucine (NMIJ CRM 6013-a), L-leucine (NMIJ CRM 6012-a), L-phenylalanine (NMIJ CRM 6014-a) and L-valine (NMIJ CRM 6015-a), and by the vibration type density meter calibrated with JCSS Accredited Density Standard Liquid. The certified values, therefore, are traceable to the International System of Units (SI) via amino acid analysis based on the primary method.

Indicative Values

The indicative values of this CRM are the amount of substance concentrations of amyloid β and total amyloid β at 20 °C, and are determined by the certified reference values and molar masses. The uncertainty of the indicative values is the expanded uncertainty obtained by multiplying the combined standard uncertainty by a coverage factor (k) of 2.45, and it is the half-width of an interval estimated to have a level of confidence of approximately 95 %.

Substance	CAS No.	Indicative value Amount of substance concentration (μmol/L)	Expanded uncertainty Amount of substance concentration (μmol/L)
Amyloid β	107761-42-2	9.5	1.6

Substance	Indicative value Amount of substance concentration (μmol/L)	Expanded uncertainty Amount of substance concentration (μmol/L)
Total amyloid β (Mixture of amyloid β, oxdated amyloid β, deamidated amyloid β, and isomerized amyloid β)	10.2	2.3

Expiration of Certification

This certificate is valid for 6 months from the date of shipment, provided that this CRM remains unopened and is stored in accordance with the instructions given in this certificate.

Description of the Material

This CRM is the form of a white lyophilized powder of synthetic peptide having a human amyloid β (1-42) sequence. Approximately 50 μg of this CRM in net volume is dispensed into a 10 mL glass vial and the vial is sealed in an aluminum-laminated plastic bag.

Instructions for Storage

This CRM should be stored at temperatures of ca. –80 °C and shieled from light.

Instructions for Use

1) The certified value as total amyloid β should be used for the analyses, e.g., amino acid analysis and the immunochemical method, which cannot differentiate between amyloid β and the modified form of amyloid β, such as oxidated and deamidated amyloid β, or amyloid β isomer.
2) The reconstituted material should be stored at 4 ˚C or below and used within 16 h, because amyloid β has aggregative properties.
3) Amyloid β may tend to be adsorbed on the surface of labware. Use of low-adsorption material is recommended for handling reconstituted materials.
4) This CRM must be reconstituted according to the following procedure.

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

1. Take out the aluminum-laminate 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-laminate 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 0.1 % ammonia solution through the rubber septum using a syringe*,**. Make sure that the difference of the masses between vials with 0.1 % ammonia solution and without 0.1 % ammonia solution at step 4 is within 0.99 g to 1.01 g.
6. Ultrasonicate the vial for 1 min to dissolve the material completel.

* The use of a microsyringe having at least 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 0.1 % ammonia solution accurately, 0.1 % ammonia solution may be ejected several times prior to reconstitution, by checking the amount of ejected water or 0.1 % ammonia solution with the balance.

** 0.1 % ammonia solution should be gravimetrically prepared by 17-fold dilution of 1 mol/L ammonia solution (equivalent to approx. 1.7 g/dL) with purified water. Total volume of diluent is recommended to be more than 2 mL. The final concentration of diluent should be ranged from 0.095 g/dL to 0.105 g/dL. At the time of certification of this CRM, 1 mol/L ammonia solution for volumetric analysis (Nacalai Tesque Co.) was purchased and used.

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

The raw material of amyloid β was synthesized and purified by Peptide Institute Inc., Osaka, Japan. This material was dissolved in 0.1 % ammonia solution 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 0.9979 g/cm^3 at 20 ˚C.

This material contains (6.5 ± 0.2) mg/L of trifluoroacetate ion, after reconstitution according to “Instruction for Use”. The numeric value after the symbol ± indicates the standard deviation of measurement.

The amino acid sequence of amyloid β is as follows:

DAEFRHDSGYEVHHQKLVFFAEDVGSNKGAIIGLMVGGVVIA

Molecular weight of amyloid β is 4514.37, and its monoisotopic mass is 4511.2696.

NMIJ Analysts

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

February 24, 2023

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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