National Institute of Advanced Industrial Science and Technology

National Metrology Institute of Japan

Reference Material Certificate

NMIJ CRM 6201-c
No. +++

C-reactive Protein Solution

This certified reference material (CRM) is recombinant human C-reactive protein solution (CRP), which is produced in accordance with the NMIJ's management system and in compliance with ISO 17034 and ISO/IEC 17025. This CRM is primarily intended for use in calibrating and controlling the precision of instruments for the determination of CRP, 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 immunoassay for quantification or value-assignment of CRP in the human serum, the commutability should be verified.

Certified Value

The certified value for the amount of substance content of monomeric CRP in 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, Amount of substance content (μmol/kg)	Expanded uncertainty, Amount of substance content (μmol/kg)
C-reactive Protein	99401-15-7	40.2	1.8

Analysis

The certified value was the weighted mean of the results of two amino acid analysis, in which the following independent hydrolysis and isotope-dilution mass spectrometry (IDMS) were used.

1) Hydrolysis was performed with vapor phase hydrochloric acid (HCl) at 130 °C for 24 h. The hydrolyzed amino acids (arginine, glutamic acid, histidine, leucine, lysine, methionine, phenylalanine, proline, and valine) were quantified by hydrophilic chromatography/mass spectrometry.
2) Hydrolysis was performed with liquid phase HCl at 150 °C for 3 h using microwave oven. The hydrolyzed amino acids (alanine, aspartic acid, glutamic acid, isoleucine, leucine, phenylalanine, proline, and valine) were quantified by reversed phase chromatography/mass spectrometry using *N*-butylnicotinic acid *N*-hydroxysuccinimide ester iodide as the derivatization reagent.

In each amino acid analysis, the amount of substance content of monomeric CRP was calculated based upon the concentration determined for each of the amino acids and the known amino acid sequence for CRP.

Metrological Traceability

The certified value is traceable to the International System of Units (SI) via amino acid analysis which was combined in part with primary method IDMS and calibrated with NMIJ CRMs (L-alanine (NMIJ CRM 6011-a), L-arginine (NMIJ CRM 6017-b), L-aspartic acid (NMIJ CRM 6027-a), L-glutamic acid (NMIJ CRM 6026-a), L-histidine (NMIJ CRM 6024-a), L-isoleucine (NMIJ CRM 6013-a), L-leucine (NMIJ CRM 6012-a), L-lysine monohydrate (NMIJ CRM 6018-a), L-methionine (NMIJ CRM 6023-a), L-phenylalanine (NMIJ CRM 6014-a), L-proline (NMIJ CRM 6016-a), L-valine (NMIJ CRM 6015-a)).

Indicative Value

The mass concentration of CRP is given in the table below as the indicative value, which was calculated using the certified value, averaged relative molecular mass obtained by mass spectrometry, and the density of this CRM at 25 °C. The uncertainty of the indicative value was calculated from the uncertainty of the certified value.

	Indicative Value, Mass concentration (g/L)	Expanded uncertainty, Mass concentration (g/L)
C-reactive Protein	0.931	0.040

Expiration of Certification

This certificate is valid until 30 September 2022, 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 clear and colorless liquid at room temperature. This CRM of ca. 2 mL in net volume is kept in a semitransparent plastic vial, and the vial is sealed in an aluminum-laminated plastic bag.

Homogeneity

The homogeneity of the CRM was determined by gel permeation chromatography for 10 vials selected from 70 vials after stratification. The uncertainty derived from inhomogeneity is reflected in the uncertainty of the certified value.

Instructions for Storage

This CRM should be stored in a clean place under refrigeration (approx. 4 °C) without allowing it frozen.

Instructions for Use

Before opening, the solution should be flopped upside down approximately ten times. This CRM should be used promptly once the vial is opened. CRP tends to be absorbed on the surface on labware. It is recommended that the use of low adsorption material and buffer containing carrier protein for the dilution of this CRM. This CRM is for laboratory use only and not for *in vivo* use.

Precautions for Handling

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

Preparation

This CRM was prepared and bottled by Oriental Yeast, Co., Ltd. using their recombinant human CRP.

Technical Information

1. Theoretical molecular mass of the CRP in this CRM is 23028.1 in consideration of the amino acid sequence and the modified structure of this CRP which is pyroglutamated at N-terminus and has one internal S-S bond. Averaged relative molecular mass obtained by mass spectrometry was 23028.2.
2. The density at 25 °C of this CRM was determined to be 1.0051 g/cm^3.
3. The solvent composition of this CRM is 20 mmol/L Tris- HCl (pH 7.5), 0.14 mol/L NaCl, 2 mmol/L $CaCl_2$ and 0.05 % NaN_3.

NMIJ Analysts

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