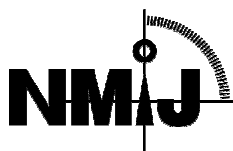


National Institute of Advanced Industrial Science and Technology

## National Metrology Institute of Japan



## Reference Material Certificate

NMIJ CRM 6201-b

No. +++

C-reactive Protein Solution



This certified reference material (CRM) is a recombinant human C-reactive protein solution (CRP). It has been produced in accordance with NMIJ's management system, and in compliance with ISO Guide 0034:2009 and ISO/IEC 17025:2005. This CRM is primarily intended for use in calibrating and controlling the precision of instruments for CRP determination, and for value-assignment of a 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 an 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 following table. The quoted uncertainty 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 ( $\mu\text{mol/kg}$ )	Expanded uncertainty ( $\mu\text{mol/kg}$ )
C-reactive protein	99401-15-7	40.0	1.6

**Analysis**

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

- 1) Hydrolysis was performed using vapor-phase hydrochloric acid (HCl) at 130 °C for 24 h. The hydrolyzed amino acids (alanine, arginine, isoleucine, leucine, lysine, phenylalanine, proline, and valine) were quantified by hydrophilic chromatography/mass spectrometry.
- 2) Hydrolysis was performed using liquid-phase HCl at 150 °C for 3 h in a microwave oven. The hydrolyzed amino acids (alanine, aspartic acid, glutamic acid, isoleucine, leucine, lysine, phenylalanine, proline, and valine) were quantified by reversed-phase chromatography/mass spectrometry performed 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 using 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 the primary method (IDMS) and calibrated with NMIJ amino acid CRMs (L-alanine (NMIJ CRM 6011-a), L-arginine (NMIJ CRM 6017-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-lysine monohydrate (NMIJ CRM 6018-a), L-phenylalanine (NMIJ CRM 6014-a), L-proline (NMIJ CRM 6016-a), and L-valine (NMIJ CRM 6015-a)).

**Indicative Value**

The mass concentration of CRP given in the following table is an indicative value, which was calculated using the certified

value, averaged relative molecular mass obtained by mass spectrometry, and the density of this CRM at 20 °C. The uncertainty of the indicative value was calculated from that of the certified value.

	Mass concentration (g/L)	Expanded uncertainty (g/L)
C-reactive protein	0.926	0.035

### Stability

The stability of the CRM at –80 °C and 4 °C was evaluated by a stability study. The uncertainty derived from stability is reflected in the uncertainty of the certified value. The stability will be monitored once a year at NMIJ.

### Expiration of Certification

The certification of this CRM is valid for six months after 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, colorless liquid at room temperature. Approximately 2 mL of the material was bottled in a semitransparent plastic vial, and the vial was kept in an aluminum-laminated bag.

### Homogeneity

The homogeneity of the CRM was determined by gel permeation chromatography, by analyzing 10 vials selected from 100 vials after stratification. The uncertainty derived from inhomogeneity is reflected in the uncertainty of the certified value.

### Precautions for Storage

This CRM should be kept in a clean place under refrigeration (about 4 °C) but should not be frozen.

### Instructions for Use

Before opening, the solution should be flopped upside down approximately ten times. Once the vial is opened, the solution should be used immediately. CRP may tend to be absorbed on the surface of labware. The use of low-adsorption material and buffer containing a carrier protein is recommended for the dilution of this CRM. Lastly, this CRM is for laboratory use only and not for *in vivo* use.

### Instructions for Handling

The CRM should be handled according to the instructions given in its safety data sheet (SDS).

### Preparation Method

This CRM was prepared from commercial recombinant human CRP supplied by Oriental Yeast Co., Ltd., which bottled the CRM.

### Information

1. The theoretical molecular mass of this CRM is 23028.1, calculated on the basis of the amino acid sequence and the modified structure of this CRM, which is pyroglutamated at *N*-terminus and has one internal S–S bond. The averaged relative molecular mass of this CRM as obtained by mass spectrometry is 23026.4.
2. The density of this CRM at 20 °C is 1.0055 g/cm<sup>3</sup>. When the solution temperature is changed from 20 °C to 4 °C, the density increases by approximately 0.2%.
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<sub>2</sub>, and 0.05% NaN<sub>3</sub>.

### NMIJ Analysts

For this CRM, the technical manager is A. Takatsu, the production manager is M. Kato, and the analysts are M. Kato, T.

Kinumi, S. Fujii, M. Yoshioka, and M. Goto.

**Technical Information**

Customer registration on the NMIJ website shown below will facilitate notification of any revision of the information given above. Technical reports regarding this CRM can also be obtained from the contact details shown below.

**Reproduction of Certificate**

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

**Additional Statement**

The certified values of CRP were validated in the program “Co-validation of C-reactive protein CRM” conducted by the ACRM (Asian Collaboration on Reference Materials) that is jointly constructed by NMIJ (Japan), KRISS (Korea), and NIM (China).

August 29, 2012

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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Note: This certificate is a translation of the original Japanese certificate and is not an official document.