NATIONAL INSTITUTE OF STANDARDS AND TECHNOLOGY
U.S. DEPARTMENT OF COMMERCE

Date of Issue:
10 June 2025

Standard Reference Material® 2924
C-Reactive Protein Solution
CERTIFICATE OF ANALYSIS

Purpose: The certified value delivered by this Standard Reference Material (SRM) is intended for calibrating procedures and devices for the determination of C-reactive protein (CRP) in human serum. It can also be used for value-assignment of calibrators and control materials.

Description: A unit of SRM 2924 consists of three vials, each containing approximately 1 mL of a solution of recombinant CRP.

Certified Value: The certified value for monomeric concentration of CRP is provided below. Metrological traceability is to the SI derived units for molar concentration (expressed as micromoles per liter).

Certified CRP concentration: 20.6 µmol/L ± 1.2 µmol/L $k = 2.15$

A NIST certified value is a value for which NIST has the highest confidence in its accuracy in that all known or suspected sources of bias have been investigated or taken into account [1]. The certified concentration was determined using higher-order reference measurement procedures [2] calibrated with high-purity amino acid standards. The uncertainty provided for the value is an expanded uncertainty about the mean to cover the measurand with approximately 95 % confidence. The expanded uncertainty is calculated as $U = ku_c$, where u_c is the combined uncertainty, and k is a coverage factor corresponding to approximately 95 % confidence [3].

Non-Certified Values: Non-certified values are provided in Appendix A.

Additional Information: Information regarding source, preparation, and analysis is provided in Appendix B.

Period of Validity: The certified value delivered by **SRM 2924** is valid within the measurement uncertainty specified until **15 May 2030**. The certified value is nullified if the material is stored or used improperly, damaged, contaminated, or otherwise modified.

Maintenance of Certified Value: NIST will monitor this SRM over the period of its validity. If substantive technical changes occur that affect the certification, NIST will issue an amended certificate through the NIST SRM website (https://www.nist.gov/srm). Before making use of any of the values delivered by this material, users should verify they have the most recent version of this documentation, available through the NIST SRM website (https://www.nist.gov/srm).

Safety: Consult the Safety Data Sheet (SDS) for hazard information.

Storage: The SRM is shipped frozen on dry ice. Upon receipt, material should be stored frozen below –50 °C until ready for use in the original unopened vial.

Use: SRM 2924 IS INTENDED FOR RESEARCH USE ONLY. Vials of the SRM to be analyzed should be removed from the freezer and allowed to stand at room temperature (20 °C to 25 °C) until thawed. After the material is thawed, it may be gently mixed and then centrifuged briefly (1000 g_n for 5 min) to clear material from the cap threads. Unused material may be stored at 4 °C for up to one week.

Michael J. Tarlov, Chief
Biomolecular Measurement Division

Steven J. Choquette, Director
Office of Reference Materials

Certificate Revision History on Page 2

REFERENCES

[1] Beauchamp, C.R.; Camara, J.E.; Carney, J.; Choquette, S.J.; Cole, K.D.; DeRose, P.C.; Duewer, D.L.; Epstein, M.S.; Kline, M.C.; Lippa, K.A.; Lucon, E.; Molloy, J.; Nelson, M.A.; Phinney, K.W.; Polakoski, M.; Possolo, A.; Sander, L.C.; Schiel, J.E.; Sharpless, K.E.; Toman, B.; Winchester, M.R.; Windover, D.; *Metrological Tools for the Reference Materials and Reference Instruments of the NIST Material Measurement Laboratory*; NIST Special Publication 260-136, 2021 edition; National Institute of Standards and Technology, Gaithersburg, MD (2021); available at https://nvlpubs.nist.gov/nistpubs/SpecialPublications/NIST.SP.260-136-2021.pdf (accessed June 2025).

[2] NCCLS; *Development of Definitive Methods for the National Reference System for the Clinical Laboratory, Approved Guideline*; NCCLS Publication NRSCL 1-A; National Committee for Clinical Laboratory Standards: Wayne, PA (1991).

[3] JCGM 100:2008; *Evaluation of Measurement Data — Guide to the expression of uncertainty in measurement*; Joint Committee for Guides in Metrology (JCGM) (2008) ; available at https://www.bipm.org/en/committees/jc/jcgm/publications (accessed June 2025); see also Taylor, B.N.; Kuyatt, C.E.; *Guidelines for Evaluating and Expressing the Uncertainty of NIST Measurement Results*; NIST Technical Note 1297; U.S. Government Printing Office: Washington, DC (1994); available at https://www.nist.gov/pml/nist-technical-note-1297 (accessed June 2025).

[4] Sniegoski, L.T.; Moody, J.R.; *Determination of Serum and Blood Densities*; Anal. Chem., Vol. 51, pp. 1577-1578 (1979).

[5] European Bioinformatics Institute; UniProt Database, Swiss Institute for Bioinformatics, and the Protein Information Resource; (P02741); available at http://www.uniprot.org (accessed June 2025).

[6] Kilpatrick, E.L.; Liao, W.-L.; Camara, J.E.; Turko, I.V.; Bunk, D.M.; *Expression and Characterization of ^{15}N-labeled Human C-reactive Protein in Escherichia Coli and Pichia Pastoris For Use in Isotope-Dilution Mass Spectrometry*; Prot. Express. Purif., Vol. 85, pp. 94–99 (2012).

[7] Program available for downloading at https://www.nist.gov/services-resources/software/nist-mass-and-fragment-calculator-software (accessed June 2025)

[8] Tanaka, T.; Horio, T.; Matuo, Y.; *Secretory Production of Recombinant Human C-reactive Protein in Escherichia Coli, Capable of Binding with Phosphorycholine and It Characterization;* Biochem. Biophys. Res. Commun., Vol. 295, pp. 163–166 (2002).

[9] Bunk, D.M.; Lowenthal, M.S.; *Isotope Dilution Liquid Chromatography-Tandem Mass Spectrometry for Quantitative Amino Acid Analysis*; Methods Mol. Biol., Vol. 828, pp. 29–38 (2012).

[10] Kato, M.; Kinumi, T.; Yoshioka, M.; Goto, M.; Fujii, S.; Takatsu, A.; *Development of C-reactive protein certified reference material NMIJ CRM 6201-b: optimization of a hydrolysis process to improve the accuracy of amino acid analysis;* Anal. Bioanal. Chem. Vol. 407, pp. 3137–3146 (2015).

Certificate Revision History: 10 June 2025 (Change of period of validity; format update; editorial revision); **10 July 2017** (Original certificate date).

Certain commercial equipment, instruments, or materials may be identified in this Certificate of Analysis to adequately specify the experimental procedure. Such identification does not imply recommendation or endorsement by the National Institute of Standards and Technology, nor does it imply that the materials or equipment identified are necessarily the best available for the purpose.

Users of this SRM should ensure that the Certificate of Analysis in their possession is current. This can be accomplished by contacting the Office of Reference Materials 100 Bureau Drive, Stop 2300, Gaithersburg, MD 20899-2300; telephone (301) 975-2200; e-mail srminfo@nist.gov; or the Internet at https://www.nist.gov/srm.

* * * * * * * End of Certificate of Analysis * * * * * * *

APPENDIX A

Non-Certified Values: The non-certified values for density, relative average molecular mass and non-certified CRP concentration (expressed in terms of grams per liter) are shown in the table below.

Non-certified values are suitable for use in method development, method harmonization, and process control but do not provide metrological traceability to the International System of Units (SI) or other higher-order reference system. All known or suspected sources of bias have not been fully investigated and are therefore provided with associated uncertainties that may reflect only measurement precision, may not include all sources of uncertainty, or may reflect a lack of sufficient statistical agreement among multiple analytical methods. The uncertainty provided with the non-certified value is an expanded uncertainty about the mean to cover the measurand with approximately 95 % confidence. The expanded uncertainty is calculated as $U = ku_c$, where u_c is the combined uncertainty, and k is a coverage factor corresponding to approximately 95 % confidence [3].

Property	Non-Certified Value			Coverage Factor, k
Density (21.7 °C)	1.0050 g/mL	±	0.0003 g/mL	2.57
CRP Relative Average Molecular Mass (dimensionless)	23 028.0	±	0.3	2.20
CRP concentration	0.474 g/L	±	0.028 g/L	2.15

Non-certified Analyses: Density measurements were performed gravimetrically using the Lang-Levy pipet method [4]. Relative average molecular mass of the monomeric form of CRP was determined using liquid chromatography/mass spectrometry (LC-MS). Measurements were performed on a high-resolution, accurate mass ion trap mass analyzer operated in positive ion mode and coupled to LC with commercial C_{18} column. The relative average molecular mass was obtained by deconvolution of the intact protein multi-charge envelope. The non-certified CRP concentration value was calculated from the Certified CRP concentration using the relative average molecular mass values. Metrological traceability of the concentration value is to the SI derived units for mass concentration (expressed as grams per liter). The theoretical molecular mass of CRP was 23 027.8 based on the reported amino acid sequence and post-translational modifications (amino-terminus pyroglutamation and a single disulfide bond) [5] as calculated using the program NIST Mass and Fragment Calculator [6, 7].

Period of Validity: The non-certified values are valid within the measurement uncertainty specified until **15 May 2030.** The value assignments are nullified if the material is stored or used improperly, damaged, contaminated, or otherwise modified.

Maintenance of Non-Certified Values: NIST will monitor this material to the end of its period of validity. If substantive technical changes occur that affect the non-certified values during this period, NIST will update this Certificate of Analysis. Before making use of any of the values delivered by this material, users should verify they have the most recent version of this documentation, available through the NIST SRM website (https://www.nist.gov/srm).

* * * * * * * * * * * End of Appendix A * * * * * * * * * * *

APPENDIX B

Source and Preparation: The recombinant human CRP was procured from OYC Americas (Andover, MA) in a buffer composed of 20 mmol/L tris buffer (pH 7.5), 140 mmol/L sodium chloride, 2 mmol/L calcium chloride and 0.05 % (m/v) sodium azide packaged in a 2 mL polypropylene vial. The recombinant CRP was expressed in *Escherichia coli* and purified by affinity binding to 6-aminohexanoic acid as described in Tanaka, et al [8]. A total of 1200 vials packed into twelve boxes were received at NIST at 4 °C. The material was frozen and kept at –80 °C following preliminary investigation showing no effects of a freeze/thaw cycle on concentration or structure.

Analysis: All analyses in the value assignment of SRM 2924 were performed at NIST.

Measurement of CRP concentration by amino acid analysis (ID-LC/MS-MS): The certified value for monomeric concentration of CRP was determined through amino acid analysis using isotope dilution liquid chromatography/tandem mass spectrometry (ID-LC/MS/MS) [9] with verification using an existing, commercially available certified reference material for CRP. The measurand is the total monomeric concentration of CRP calculated using the amount-of-substance determined for each of the amino acids and the known amino acid sequence for CRP.

Samples of SRM 2924 were combined with isotope-labeled analogs of phenylalanine, proline, isoleucine, leucine, and valine and were hydrolyzed with vapor-phase hydrochloric acid (HCl) for 48 h at approximately 118 °C in sealed vessels. After hydrolysis, the samples were lyophilized and then reconstituted with 0.1 mL/L formic acid in water. Amino acids were separated using gradient-elution mixed-mode chromatography on a reverse-phase analytical column with embedded acidic ion-pairing groups. Measurements were performed on a triple quadrupole mass spectrometer, monitoring a specific transition for each amino acid. The measurements were calibrated using amino acid standards whose purity was assessed at NIST. Based upon the known amino acid sequence for CRP [5], the concentration of CRP was calculated as the mean of the concentrations determined for CRP by each of the amino acids. Analysis of SRM 2924 was performed in four independent groups containing six replicates on four separate days. Confirmation of the analysis was obtained by use of a commercially available certified reference material, NMIJ CRM6201-b, C-reactive protein solution [10], which was run in duplicate on each day of analysis. The mean value of the CRM6201-b concentration was 38.9 µmole/kg ± 0.26 µmole/kg (standard deviation) and was within the certified 95 % confidence range reported as 38.4 µmole/kg to 41.6 µmole/kg for CRM6201-b thus verifying the amino acid concentration analysis method for SRM 2924.

Homogeneity Analysis: The concentration homogeneity assessment was made at the time the certification analyses were performed. A stratified sampling plan was devised to test for homogeneity across the lot of vials. Differences were statistically identified depending from which box the sample was taken and the day of analysis. However, the magnitude of the effects on concentration were small and have been included in the overall uncertainty of the mean.

Structural Heterogeneity: Heterogeneity was assessed using size exclusion chromatography monitoring fluorescent response (excitation – 295 nm / emission – 350 nm). The use of known molecular mass standards established the presence of a principal peak (>99.5 % abundance) at the expected retention time for the pentameric form of CRP indicating heterogeneity was negligible.

* * * * * * * * * * End of Appendix B * * * * * * * * * *