National Institute of Standards and Technology
U.S. Department of Commerce

Date of Issue:
24 November 2021

Standard Reference Material® 2926
Recombinant Human Insulin-like Growth Factor 1 (Frozen)
CERTIFICATE OF ANALYSIS

Purpose: This Standard Reference Material (SRM) is primarily intended for use in calibrating mass spectroscopy-based procedures and devices for the determination of Insulin-like Growth Factor 1 (IGF-1) in human serum. It can also be used for value-assignment of control materials.

Description: A unit of SRM 2926 consists of three vials, each containing approximately 0.25 mL of a solution of recombinant human IGF-1.

Certified Value: The certified value for the concentration of IGF-1was determined through amino acid analysis using isotope dilution liquid chromatography/tandem mass spectrometry (ID-LC/MS/MS) [1]. The measurand is the total concentration of IGF-1 calculated using the amount-of-substance determined for each of the measured amino acids and the known amino acid sequence for IGF-1. Metrological traceability is to the SI derived units for molar concentration (expressed as nanomoles per gram).

Certified IGF-1 concentration: 39.7 nmol/g ± 0.8 nmol/g $k = 2$

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 [2]. The certified concentration was determined using higher-order reference measurement procedures [3] calibrated with amino acid certified reference materials. 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 [4].

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

Additional Information: Additional information is provided in Appendix B.

Period of Validity: The certification of **SRM 2926** is valid, within the measurement uncertainty specified, until **24 January 2025**. The certified values are nullified if the material is stored or used improperly, damaged, contaminated, or otherwise modified.

Maintenance of Certified Value: NIST will monitor this SRM to the end of the period of validity. If substantive technical changes occur that affect the certification before the expiration of this certificate, NIST will notify the purchaser. Registration (see attached sheet or register online) will facilitate notification.

Michael J. Tarlov, Chief
Biomolecular Measurement Division

Steven J. Choquette, Director
Office of Reference Materials

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Safety: SRM 2926 IS INTENDED FOR RESEARCH USE ONLY.

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

Use: 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 to bring the material to the bottom of the tube prior to removal of any material.

Source and Preparation: The recombinant human IGF-1 was procured from PeproTech (Rocky Hill, NJ) as a solid. The recombinant human IGF-1 was expressed in *Escherichia coli*. A bulk solution of IGF-1 was prepared at NIST using an aqueous buffer consists of 40 mmol/L sodium phosphate and 35 mmol/L sodium acetate, pH 4.35. The bulk IGF-1 solution was aliquotted at NIST into approximately 1100 sterile polypropylene vials, each containing approximately 0.25 mL of IGF-1 solution. The SRM was frozen and stored at –80 °C at NIST.

Analysis: All analyses in the value assignment and characterization of SRM 2926 were performed at NIST.

Measurement of total IGF-1 concentration by amino acid analysis (ID-LC-MS/MS): The amino acid analysis method involved isotope dilution with liquid chromatography (ID-LC-MS/MS) [1]. Samples of SRM 2926 were combined with isotope-labeled analogs of arginine, leucine, proline, and valine and were hydrolyzed with vapor-phase hydrochloric acid (HCl) for approximately 24 h at approximately 120 °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 certified reference materials from the National Metrology Institute of Japan (Tsukuba, Japan). Based on the known amino acid sequence of human IGF-1 [5], the concentration of IGF-1 was calculated as the mean of the concentrations determined for IGF-1 by each of the four amino acids. Analysis of SRM 2926 was performed in three independent groups, each containing four process replicates from three different vials of the SRM.

Homogeneity Analysis: Heterogeneity 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. There was no apparent trend in the IGF-1 concentration data when plotted against the sequence in which the vials were prepared.

REFERENCES

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

[2] 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 (NIST SP) 260-136, 2021 edition; U.S. Government Printing Office: Washington, DC (2021); available at https://nvlpubs.nist.gov/nistpubs/SpecialPublications/NIST.SP.260-136-2021.pdf (accessed Nov 2021).

[3] 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).

[4] JCGM 100:2008; *Evaluation of Measurement Data — Guide to the Expression of Uncertainty in Measurement* (GUM 1995 with Minor Corrections); Joint Committee for Guides in Metrology (2008); available at http://www.bipm.org/utils/common/documents/jcgm/JCGM_100_2008_E.pdf (accessed Nov 2021); 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 Nov 2021).

[5] European Bioinformatics Institute; UniProt Database, Swiss Institute for Bioinformatics, and the Protein Information Resource; (P05019); available at http://www.uniprot.org (accessed Nov 2021).

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

[7] Sander, L.C.; Sniegoski, L.T.; *Determination of Liquid Density*; Tutorials in Analytical Chemistry, NIST Video (2016) available at https://www.nist.gov/video/determination-liquid-density (accessed Nov 2021).

[8] Program *NIST Mass and Fragment Calculator* available for downloading at https://www.nist.gov/services-resources/software/nist-mass-and-fragment-calculator-software (accessed Nov 2021).

Certificate Revision History: 24 November 2021 (Correction to reference 5; updated format, editorial changes); **24 January 2020** (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/COA/RMIS 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.

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APPENDIX A

Non-Certified Values: 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. The non-certified values of the density, monoisotopic relative molecular mass, and the non-certified concentration of the IGF-1 in SRM 2926 (expressed in terms of grams per liter) are listed in Table 1. The non-certified values for density and monoisotopic relative molecular mass were determined by the Lang-Levy pipet method [6,7] and mass spectrometry, respectively. The non-certified concentration, expressed in terms of grams per liter, was calculated using the average relative molecular mass (calculated from the amino acid sequence) and the certified IGF-1 concentration value above.

Table A1. Additional Non-Certified Values for Properties of SRM 2926

Property	Non-Certified Value			Coverage Factor, k
Density (21.7 °C):	1.0050 g/mL	±	0.0016 g/mL	2
Monoisotopic Relative Molecular Mass: (dimensionless)	7643.557	±	0.008	2
IFG-1 concentration:	0.305 g/L	±	0.006 g/L	2

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 [4].

Non-certified Analyses: Density measurements were performed gravimetrically using the Lang-Levy pipet method [6,7]. Metrological traceability of the density value is to the SI units for grams per milliliter. The monoisotopic relative molecular mass of the IGF-1 in the SRM was determined using liquid chromatography coupled to mass spectrometry (LC-MS). Measurements were performed on a high-resolution, accurate mass time-of-flight mass analyzer operated in positive ion mode and coupled to reverse-phase LC using a commercial C18 column. The monoisotopic relative molecular mass was calculated from the masses of monoisotopic peaks of the IGF-1 multiply-charged molecular ions. The theoretical monoisotopic relative molecular mass of human IGF-1, calculated from the reported amino acid sequence [5] and known post-translational modifications is 7643.586 using the program NIST Mass and Fragment Calculator [8]. The non-certified IGF-1 concentration value was calculated from the certified IGF-1 concentration using the average relative molecular mass value calculated from the reported amino acid sequence [5] and known post-translational modifications using the program NIST Mass and Fragment Calculator [8].

Period of validity: The non-certified values are valid within the measurement uncertainty specified until **24 January 2025**. 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 Appendix. Before making use of any of the values delivered by this material, users should obtain the most recent version of this documentation, available free of charge through the https://www.nist.gov/srm website.

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APPENDIX B

Overall direction and coordination for technical measurements leading to the certification were performed by D.M. Bunk of the NIST Biomolecular Measurement Division. Additional technical guidance was provided by K.W. Phinney of the Biomolecular Measurement Division.

Statistical analysis was provided by N.F. Zhang of the NIST Statistical Engineering Division.

Acquisition of the material and certification measurements were performed by D.M. Bunk.

Support aspects involved with the issuance of this SRM were coordinated through the NIST Office of Reference Materials

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