



Certificate of Analysis

Standard Reference Material[®] 2926

Recombinant Human Insulin-like Growth Factor 1 (Frozen)

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

Certified Concentration Value: The certified value for the concentration of IGF-1 was 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: $39.7 \text{ nmol/g} \pm 0.8 \text{ nmol/g}$ $k = 2$

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].

Reference Density, Monoisotopic Relative Molecular Mass Value and Concentration Values: The reference values of the density, monoisotopic relative molecular mass, and the reference concentration of the IGF-1 in SRM 2926 (expressed in terms of grams per liter) are listed in Table 1. The reference values for density and monoisotopic relative molecular mass were determined by the Lang-Levy pipet method [5,6] and mass spectrometry, respectively. The reference 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.

NIST reference values are non-certified values that represent the best estimate of the true values based on available data. 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 reference 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].

Expiration of Certification: The certification of **SRM 2926** is valid, within the measurement uncertainty specified, until **24 January 2025**, provided the SRM is handled and stored in accordance with instructions given in this certificate (see "Instructions for Storage and Use"). This certification is nullified if the SRM is damaged, contaminated, or otherwise modified.

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.

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Certificate Issue Date: 24 January 2020

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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.

Maintenance of SRM Certification: NIST will monitor this SRM over the period of its certification. 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.

NOTICE AND WARNING TO USERS

Warning: SRM 2926 IS INTENDED FOR RESEARCH USE ONLY.

INSTRUCTIONS FOR STORAGE AND USE

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, PREPARATION, AND ANALYSIS

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 [7], 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.

Reference Analyses: Density measurements were performed gravimetrically using the Lang-Levy pipet method [5,6]. 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 [7] and known post-translational modifications is 7643.586 using the program NIST Mass and Fragment Calculator [8]. The reference 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 [7] and known post-translational modifications using the program NIST Mass and Fragment Calculator [8].

Table 1. Additional Reference Values for Properties of SRM 2926

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

REFERENCES

- [1] 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).
- [2] May, W.; Parris, R.; Beck II, C.; Fassett, J.; Greenberg, R.; Guenther, F.; Kramer, G.; Wise, S.; Gills, T.; Colbert, J.; Gettings, R.; MacDonald, B.; *Definition of Terms and Modes Used at NIST for Value-Assignment of Reference Materials for Chemical Measurements*; NIST Special Publication 260-136 (2000); available at <https://www.nist.gov/system/files/documents/srm/SP260-136.PDF> (accessed Jan 2020).
- [3] NCCLS; *Development of Definitive Methods for the National Reference System for the Clinical Laboratory, Approved Guideline*; NCCLS Publication NRSLC 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 Jan 2020); 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 Jan 2020).
- [5] Sniegowski, L.T.; Moody, J.R.; *Determination of Serum and Blood Densities*; Anal. Chem., Vol 51, pp. 1577–1578 (1979).
- [6] Sander, L.C.; Sniegowski, L.T.; *Determination of Liquid Density*; Tutorials in Analytical Chemistry, NIST Video (2016) available at <https://www.nist.gov/video/determination-liquid-density> (accessed Jan 2020)
- [7] European Bioinformatics Institute; UniProt Database, Swiss Institute for Bioinformatics, and the Protein Information Resource; (P02741); available at <http://www.uniprot.org> (accessed Jan 2020).
- [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 Jan 2020).

Users of this SRM should ensure that the Certificate of Analysis in their possession is current. This can be accomplished by contacting the SRM Program: telephone (301) 975-2200; fax (301) 948-3730; e-mail srminfo@nist.gov; or via the Internet at <https://www.nist.gov/srm>.