



Certificate of Analysis

Standard Reference Material[®] 2927

¹⁵N-Labeled Recombinant Human Insulin-like Growth Factor 1

(Frozen)

This Standard Reference Material (SRM) is a solution of recombinant human insulin-like growth factor 1 (IGF-1) which has been enriched with nitrogen-15 (¹⁵N). This SRM is intended primarily for use as an internal standard for stable isotope dilution liquid chromatography mass spectrometry methods used for the quantification of human IGF-1 [1]. A unit of SRM 2927 consists of three vials, each containing approximately 50 microliters of ¹⁵N-labeled recombinant human IGF-1 solution.

Certified Value: 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 level of ¹⁵N incorporation into the recombinant human IGF-1 was determined through measurement of the relative molecular masses and the isotopic distributions of the protein molecular ions by liquid chromatography coupled to mass spectrometry (LC-MS). The measurand is the degree of nitrogen-15 incorporation into the chemical structure of the recombinant human IGF-1. Metrological traceability is to the SI unit of mass, expressed as a percentage.

Certified ¹⁵N incorporation: 98.72 % ± 0.10 % $k = 2$

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 the coverage factor corresponding to approximately 95 % confidence [3].

Reference Values: A NIST reference value is a non-certified value that is the best estimate of the true value based on available data; however, the value does not meet the NIST criteria for certification and is provided with associated uncertainties that may reflect only measurement reproducibility, may not include all sources of uncertainty, or may reflect a lack of sufficient statistical agreement among multiple analytical methods [2]. The reference values are listed in Table 1. The measurands are the values of relative molecular mass, and the concentration of the ¹⁵N-labeled recombinant human IGF-1, as determined by the methods used. Values are metrologically traceable to the measurement procedures as indicated.

Expiration of Certification: The certification of **SRM 2927** is valid, within the measurement uncertainty specified, until **24 April 2025**, provided the SRM is handled and stored in accordance with the instructions given in this certificate (see “Instructions for Storage and Use”). This certification is nullified if the SRM is damaged, contaminated, or otherwise modified.

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.

Overall direction and coordination of 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.

Michael J. Tarlov, Chief
Biomolecular Measurement Division

Gaithersburg, MD 20899
Certificate Issue Date: 28 July 2020

Steven J. Choquette, Director
Office of Reference Materials

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

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

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

NOTICE AND WARNING TO USERS

SRM 2927 IS INTENDED FOR RESEARCH USE ONLY. SRM 2927 has been obtained from bacteria cells and has the potential to contain toxins that may pose a health risk. Normal caution and care should be exercised during the material's handling and use.

INSTRUCTIONS FOR STORAGE AND USE

Storage: The SRM is shipped frozen on dry ice, in polypropylene vials. Upon receipt, materials should be stored in the original unopened vial and kept frozen below $-50\text{ }^{\circ}\text{C}$ until ready for use.

Use: Vials of the SRM to be used should be removed from the freezer and allowed to stand at room temperature ($20\text{ }^{\circ}\text{C}$ to $25\text{ }^{\circ}\text{C}$) 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 vial prior to removal of any material.

SOURCE, PREPARATION, AND ANALYSIS⁽¹⁾

The ^{15}N -labeled recombinant human IGF-1 solution was obtained from Ajinomoto North American, Inc. (Raleigh, NC). The ^{15}N -labeled recombinant human IGF-1 was expressed in *Corynebacterium glutamicum*. The ^{15}N -labeled recombinant human IGF-1 solution is an aqueous buffer and consists of approximately 40 mmol/L sodium phosphate and approximately 35 mmol/L sodium acetate, pH 4.35. The bulk ^{15}N -labeled recombinant human IGF-1 solution was aliquoted at NIST into approximately 700 sterile polypropylene vials, each containing approximately 0.05 mL of protein solution. The SRM was frozen and stored at $-80\text{ }^{\circ}\text{C}$ at NIST. All analyses in the value assignment and characterization of SRM 2927 were performed at NIST.

Measurement of the ^{15}N incorporation by LC-MS: For certification of the ^{15}N -incorporation level into the recombinant human IGF-1, the intact protein was analyzed by LC-MS and the isotopic distributions of the protein molecular ions were evaluated [4]. Measurements were performed on a high-resolution, accurate mass time-of-flight mass analyzer operated in positive ion mode and coupled to reversed-phase LC using a commercial C18 column. Measurements of the multiply-charged molecular ions of the protein were performed in full-scan mode. The measured isotopic distributions of the ^{15}N -labeled recombinant human IGF-1 molecular ions were compared to theoretical isotopic distributions using the NIST Isotopic Enrichment Calculator (beta version 1.4) [5] to determine the degree of ^{15}N incorporation into the recombinant human IGF-1.

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 ^{15}N -incorporation data when plotted against the sequence in which the vials were prepared.

Reference Analyses: The measured relative molecular mass of the ^{15}N -labeled recombinant human IGF-1 in the SRM was determined using LC-MS. Measurements were performed on a high-resolution, accurate mass time-of-flight mass analyzer operated in positive ion mode and coupled to reversed-phase LC using a commercial C18 column. The measured relative molecular mass was calculated from the masses of peaks of the ^{15}N -labeled recombinant human IGF-1 multiply-charged molecular ions. The reference concentration of the ^{15}N -labeled recombinant human IGF-1 was determined using LC-MS to transfer the reference concentration of unlabeled recombinant human IGF-1 in a standard reference material [6] to SRM 2927. The LC-MS method measured signals from the $(\text{M}+6\text{H})^{6+}$ molecular ions from both the ^{15}N -labeled and unlabeled recombinant human IGF-1 in selected ion monitoring (SIM) mode. Samples were prepared by blending aliquots of both the standard and SRM 2927 in approximately equal parts. The average measured peak area ratio of the unlabeled to ^{15}N -labeled $(\text{M}+6\text{H})^{6+}$ molecular ion was calculated and used to calculate the reference concentration of SRM 2927.

⁽¹⁾ Certain commercial equipment, instruments or materials are identified in this certificate 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.

Table 1. Reference Values for SRM 2927

Property	Reference Value	Units	Coverage Factor, <i>k</i>
Relative Molecular Mass	7733.30 ± 0.04	dimensionless	2
¹⁵ N-labeled IGF-1 concentration	0.285 ± 0.007	g/L	2

REFERENCES

- [1] Cox, H.D.; Lopes, F.; Woldemariam, G.A.; Becker, J.O.; Parkin, M.C.; Thomas, A.; Butch, A.W.; Cowan, D.A.; Thevis, M.; Bowers, L.D.; Hoofnagle, A.N.; *Interlaboratory Agreement of Insulin-like Growth Factor 1 Concentrations Measured by Mass Spectrometry*; Clin. Chem., Vol. 60, pp. 541–548 (2014).
- [2] May, W.; Parris, R.; Beck, C.; Fassett, J.; Greenberg, R.; Guenther, F.; Kramer, G.; Wise, S.; Gills, T.; Colbert, J.; Gettings, R.; MacDonald, B.; *Definitions of Terms and Modes Used at NIST for Value-Assignment of Reference Materials for Chemical Measurements*; NIST Special Publication 260-136; U.S. Government Printing Office: Washington, DC (2000); available at <https://www.nist.gov/system/files/documents/srm/SP260-136.PDF> (accessed Jul 2020).
- [3] 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 (JCGM) (2008); available at https://www.bipm.org/utis/common/documents/jcgm/JCGM_100_2008_E.pdf (accessed Jul 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 Jul 2020).
- [4] MacCoss, M.J.; Wu, C.C.; Matthews, D.E.; Yates, J.R.; *Measurement of the Isotope Enrichment of Stable Isotope-Labeled Proteins using High-Resolution Mass Spectra of Peptides*; Anal. Chem., Vol. 77, pp. 7646–7653 (2005).
- [5] National Institute of Standards and Technology, *Isotope Enrichment Calculator*; available at <https://www.nist.gov/services-resources/software/isotope-enrichment-calculator> (accessed Jul 2020).
- [6] SRM 2926; *Recombinant Human Insulin-like Growth Factor 1 (Frozen)*; National Institute of Standards and Technology; U.S. Department of Commerce: Gaithersburg, MD (24 January 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>.