Supelco®

www.sigmaaldrich.com

Certified Reference Material - Certificate of Analysis

IGF-1, Primary Measurement Standard

Insulin-like Growth Factor 1 (Human Recombinant)

Product No.: I-033-1ML
Lot No.: FN11062013
Description of CRM: IGF-1 in 2 mM Methionine with 2% Acetic acid in Water (v/v) (Solution)
Expiration Date: February 2023 See Section "Stability Assessment".
Storage: Store unopened in freezer (-10 °C to -25 °C).
Shipping: Dry Ice. See Section "Stability Assessment".
CAS No.: 67763-96-6

Analyte	Certified Concentration ± associated uncertainty U, U=(k)x(u); (k=2)
Total IGF-1 (unmodified and modified forms) [1]	**122.87 ± 4.37 µg/mL**
Native IGF-1 (unmodified form) [2]	**120.17 ± 4.27 µg/mL**

[1] The total IGF-1 content is determined by quantitative amino acid analysis (AAA) as total protein content. Known modifications include oxidized, disulfide scrambled, and deamidated IGF-1.

[2] The native IGF-1 content is determined by quantitative AAA and chromatographic purity. Native IGF-1 is the main isoform of IGF-1 as determined by HPLC/UV/MS.

Metrological traceability: Traceable to the SI and higher order standards from NIST through an unbroken chain of comparisons. See "Details on metrological traceability" on page 2.

Measurement method: The certified value is assigned by validated AAA and HPLC/UV/MS methods. The certified property value is determined from analytical results obtained from samples pulled from across the lot. See "Details about certification process" on page 2.

Intended use: This Certified Reference Material is suitable for the in vitro identification, calibration, and quantification of the analyte(s) in analytical and R&D applications. Not suitable for human or animal consumption.

Minimum sample size: 10 µL for quantitative applications

Instructions for handling and correct use: IGF-1 content is determined by quantitative amino acid analysis (AAA) and HPLC/UV/MS. No adjustment required before use.

Thaw at refrigerator or room temperature and mix well before use. Do not refreeze product once thawed. Thawed product may be stored refrigerated for 8 weeks. Users should quantitatively transfer desired volume using established good laboratory practices to spike into matrix or to dilute to the desired concentration. Each ampoule is intended for one-time use.

Health and safety information: Please refer to the Safety Data Sheet for detailed information about the nature of any hazard and appropriate precautions to be taken.

Accreditation: Cerilliant Corp. is accredited by the US accreditation authority ANAB as registered reference material producer AR-1353 in accordance with ISO 17034 and registered testing laboratory AT-1352 according to ISO/IEC 17025.

ANAB ACCREDITED ISO 17034 REFERENCE MATERIAL PRODUCER

Darren Ellsworth, Quality Assurance Manager

October 25, 2023
Issue Date

Cerilliant Corporation, 811 Paloma Drive, Suite A Round Rock,
TX 78665, USA, Tel: 800-848-7837 / 512-238-9974; www.cerilliant.com

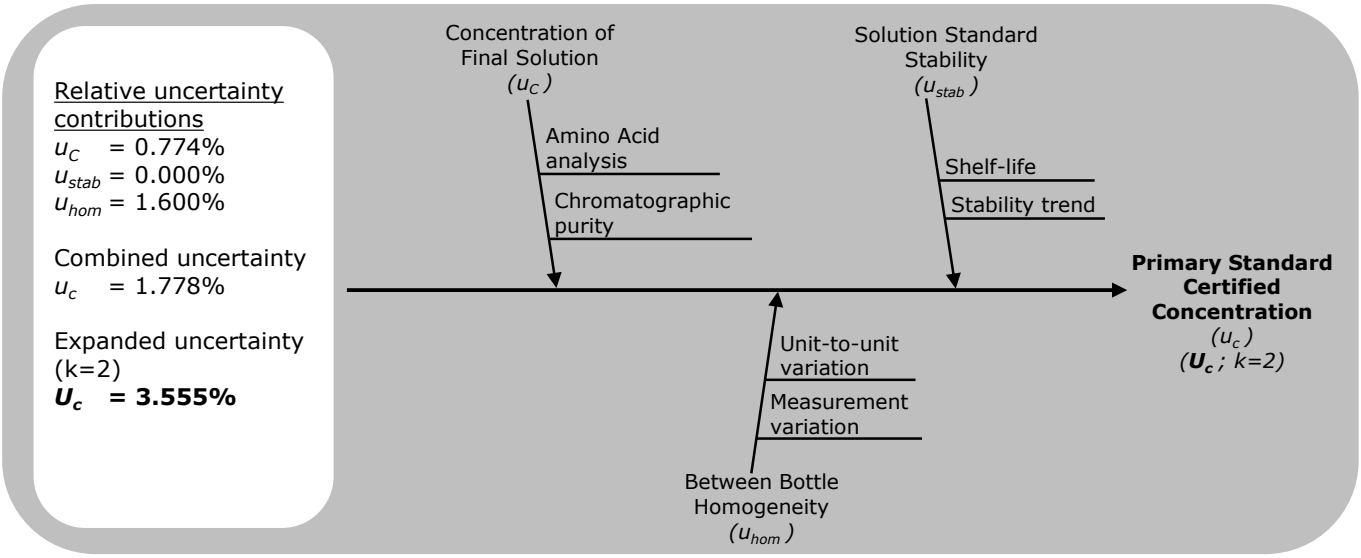
Packaging: 2 mL amber USP Type I glass ampoule containing not less than 1 mL of certified solution. Ampoules are overfilled to ensure a minimum 1 mL volume can be transferred when using a 1 mL Class A volumetric pipette..

Details on starting materials: Each raw material utilized has been identified and thoroughly characterized through the use of multiple analytical techniques. Spectral data is provided on subsequent pages of this CoA.

Certificate of Origin: The human insulin in this product is of recombinant origin. This material is a product of Korea.

Associated uncertainty:

The uncertainty has been calculated by statistical analysis of all aspects of our production system and incorporated uncertainty of the AAA and HPLC/UV/MS methods and between bottle homogeneity. Uncertainty components of the Primary Standard concentration are shown in the figure below. Uncertainty is expressed as an expanded uncertainty in accordance with ISO 17034 at the approximate 95% confidence interval using a coverage factor of k=2. Stability uncertainty was determined to be negligible by trend analysis.



Details on metrological traceability:

- This standard has been gravimetrically prepared using balances that have been fully qualified and calibrated to ISO 17025 requirements. All calibrations utilize NIST traceable weights which are calibrated externally by a qualified ISO 17025 accredited calibration laboratory to NIST standards. Qualification of each balance includes the assignment of a minimum weighing by a qualified and ISO 17025 accredited calibration vendor taking into consideration the balance and installed environmental conditions to ensure compliance with USP tolerances of NMT 0.10% relative error.
- Fill volume to predetermined specifications is gravimetrically verified throughout the dispensing process using qualified and calibrated balances.
- The density and material certification is traceable to the SI and higher order reference materials through mass measurement and instrument qualification and calibrations. Traceability has been established through calibration of the certification techniques (AAA, HPLC/UV/MS).

Details about certification process:

This standard has been prepared and certified under the ISO 17034, ISO/IEC 17025, and ISO 9001 standards. This standard meets the requirements of a Certified Reference Material and a Primary Standard as defined by ISO and is traceable to the SI and higher order standards through an unbroken chain of comparisons.

- Nominal concentration is calculated based on: total protein content determined by quantitative amino acid analysis (AAA) and total and unmodified IGF-1 chromatographic purity by HPLC/UV/MS.
- Fill volume is gravimetrically verified throughout the dispensing process using qualified and calibrated balances.
- Additional certification information available upon request.

Solution Standard Value Assignment and Homogeneity

The total and unmodified IGF-1 Content is determined with quantitative AAA and HPLC/UV/MS Chromatographic Purity. Within- and between-bottle homogeneity are analytically verified by quantitative AAA.

Material Name:	IGF-1	**CAS Number:**	67763-96-6
Solution Standard Lot:	FN11062013	**Molecular Weight:**	7.6 kDa
Raw Material Lot:	PN03201702		

Analytical Test	Method	Results	%RSD - Homogeneity
Protein Content by Amino Acid Analysis	88167067-LS [1,2]	122.87 µg/mL	1.6
Protein ID by Intact Mass	89566999-LS [1,2]	Consistent with Protein Sequence	NA
Oxidized IGF-1 and IGF-1 Isoform Content by HPLC/UV/MS	89566999-LS [1,2]	0.11% IGF-1 Isoform 1.30% Oxidized IGF-1	NA
Unmodified IGF-1 Chromatographic Purity by HPLC/UV/MS	89566999-LS [1,2]	97.8%	NA

[1] Validated analytical method

[2] Facility performing analytical testing located at 2909 Laclede Avenue, Saint Louis, MO 63103.

Spectral and Physical Data

HPLC/UV/MS

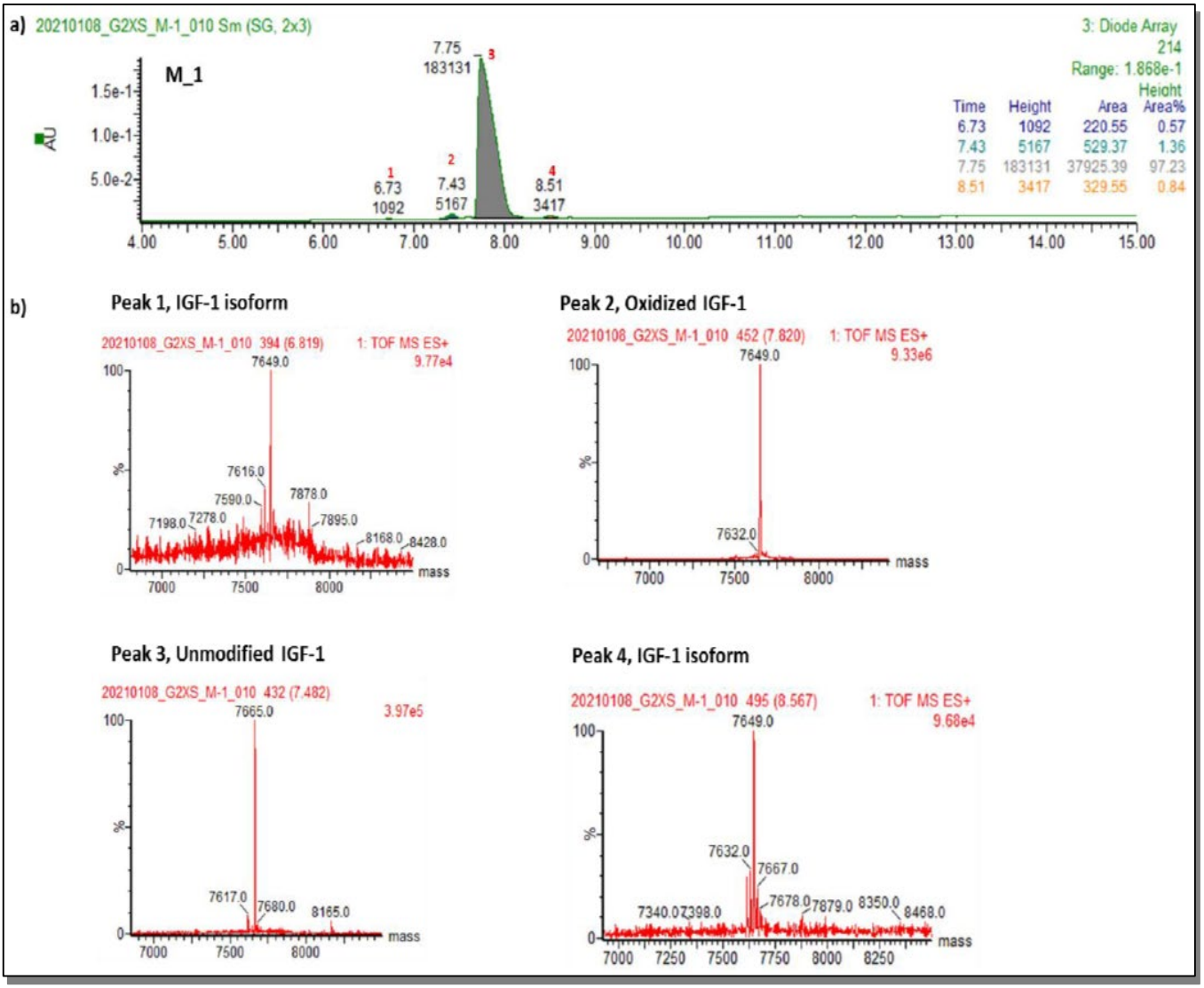
Column: BIOshell A400 Protein C4™, 3.4 µm, 2.1 x 150 mm

Mobile Phase: A: 0.1% Trifluoroacetic acid in Water
B: 0.1% Trifluoroacetic acid in Acetonitrile

Gradient:

Time (min)	%A	%B
0.0	75	25
20.0	65	35
20.5	20	80
22.5	20	80
23.0	75	25
35.0	75	25

Flow Rate: 500 µL/min
Wavelength: 214 nm
Scan Range: 300-4000 amu
Ionization: Electrospray, Positive Ion
Instrument: Waters Xevo G2XS Q-ToF
Acquired: January 08, 2021



a) UV_{214} chromatogram

b) Deconvoluted mass spectra for IGF-1 isoform (peaks 1 and 4), oxidized IGF-1 (peak 2), and unmodified IGF-1 (peak 3).

Stability

Short term stability studies have been performed under accelerated conditions for a period of up to four weeks. Short term data is utilized to predict long term stability and to support transport conditions and normal laboratory use. Real-time stability studies are performed at the recommended storage conditions over the life of the product.

Short Term Stability: *A summary of accelerated stability findings is listed below.*

Storage Condition	Mean Kinetic Temperature (MKT)	Time Period/Result
Freezer	-15°C	No decrease in purity was noted after four weeks.
Refrigerator	4°C	
Room Temperature	21°C	
40°C	40°C	6.28% decrease in purity was noted after two weeks.

Transport/Shipping: *Ship Frozen on Dry Ice.*

Short Term Storage: *Stability data supports short term storage for up to 2 months at Refrigerate conditions.*

Long Term Stability: *Long term stability has been assessed for Freezer storage (-10 °C to -25 °C) conditions. Stability of a minimum of 14 months has been established through real-time stability studies.*

Commutability

This standard is a solution of a pure substance in an aqueous solvent and is a Primary Standard. This Primary Standard is suitable for use in the preparation of calibrators and/or controls in any biological matrix. This standard is not in a biological matrix and therefore commutability to methods or standards in biological matrices does not apply.

COA Revision History

Revision No.	Date	Reason for Revision
00	February 04, 2021	Initial version
01	March 07, 2022	Added Long Term Stability Section
		Revised Retest Date from February 2022 to February 2023
02	October 25, 2023	Changed Retest Date of February 2023 to Expiration Date of February 2023.