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Certified Reference Material - Certificate of Analysis

Adalimumab (Humira), Primary Measurement Standard

Product No.:	A-166-0.25ML
Lot No.:	FN04112201
Description of CRM:	Adalimumab (Humira) in 12.5 mM Histidine buffer (solution)
Retest Date:	June 2025 See Stability Section
Storage:	Store unopened in freezer (-10 °C to -25 °C).
Shipping:	Dry Ice. See Stability Section
Chemical formula:	NA
CAS No.:	331731-18-1

Analyte	Certified Concentration ± associated uncertainty U, U=(k)x(u); (k=2)
Adalimumab (Humira) [1]	**9.6 ± 0.6 mg/mL**

[1] The total Adalimumab (Humira) content is determined by quantitative amino acid analysis (AAA).

♦ The density of the solution is 1.0013 g/mL.

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 method. The certified property value is determined from analytical results obtained from samples pulled from across the lot. See "Details about certification process" on page 3.

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: 20 µL for quantitative applications

Instructions for handling and correct use: The Adalimumab (Humira) content is determined by quantitative AAA. No adjustment required before use.

Thaw at refrigerator or room temperature and mix well before use. Do not refreeze product once thawed.

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 DIN EN ISO/IEC 17025.

ANAB ACCREDITED ISO 17034 REFERENCE MATERIAL PRODUCER

James Schmitt, Quality Assurance Manager

April 16, 2025
Issue Date

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TX 78665, USA, Tel: 800-848-7837 / 512-238-9974; www.cerilliant.com
Sigma-Aldrich Production GmbH is a subsidiary of Merck KGaA, Darmstadt, Germany.

Packaging: 2 mL amber USP Type I glass ampoule containing not less than 0.25 mL of certified solution. Ampoules are overfilled to ensure a minimum 0.25 mL volume can be transferred.

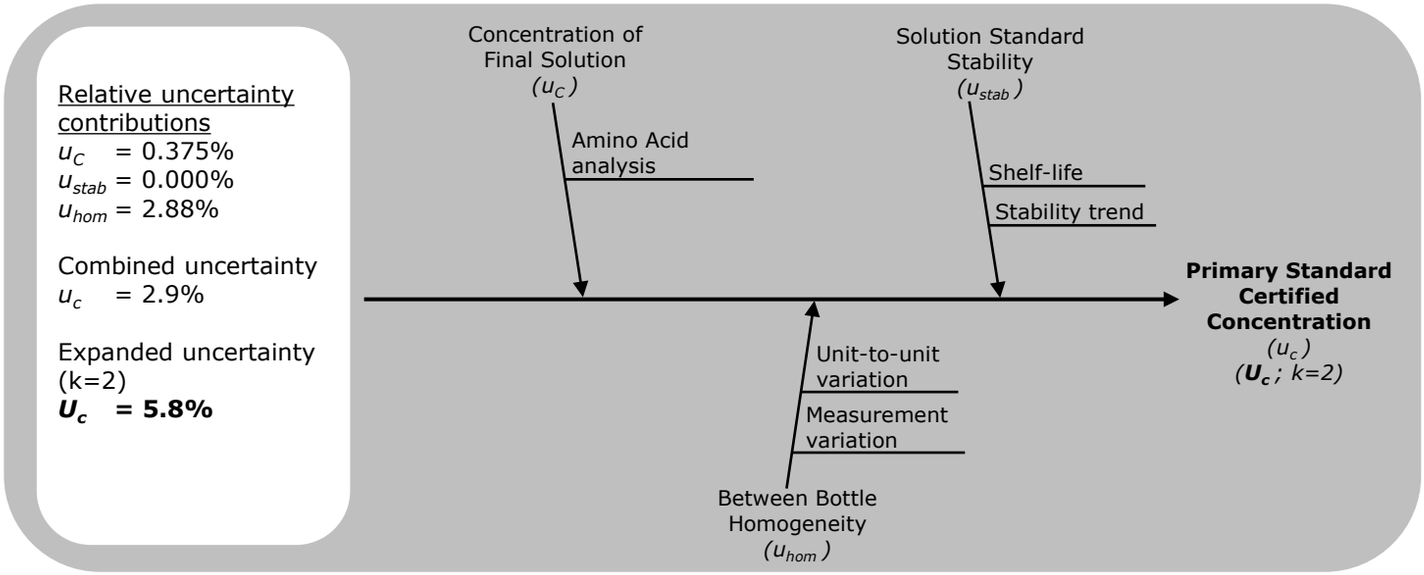
Details on starting materials: Each raw material utilized has been identified and thoroughly characterized through the use of multiple analytical techniques. Spectral data is provided on subsequent pages of this CoA.

Certificate of Origin: The Adalimumab (Humira) in this product is of synthetic origin.

Country of Origin: United States of America

Associated uncertainty:

The uncertainty has been calculated by statistical analysis of all aspects of our production system and incorporated uncertainty of the AAA 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 calcuated based on: Total protein content determined by quantitative amino acid analysis (AAA).
- 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

Adalimumab (Humira) content is determined with quantitative AAA. Within- and between-bottle homogeneity are analytically verified by quantitative AAA.

Material Name:	Adalimumab (Humira)	**Chemical Formula:**	NA
Solution Standard Lot:	FN04112201	**CAS Number:**	331731-18-1
Raw Material Lot:	PN02282204	**Molecular Weight:**	148080 Da

Material Characterization Summary

Analytical Test	Method	Results (mg/mL)	%RSD - Homogeneity
Protein Content by Amino Acid Analysis	121814979-LS [1]	9.6	2.9
Protein ID by Amino Acid Analysis	122645724-LS [1]	Consistent with Protein Sequence	NA

[1] Validated analytical method

Spectral and Physical Data

HPLC/UV/MS

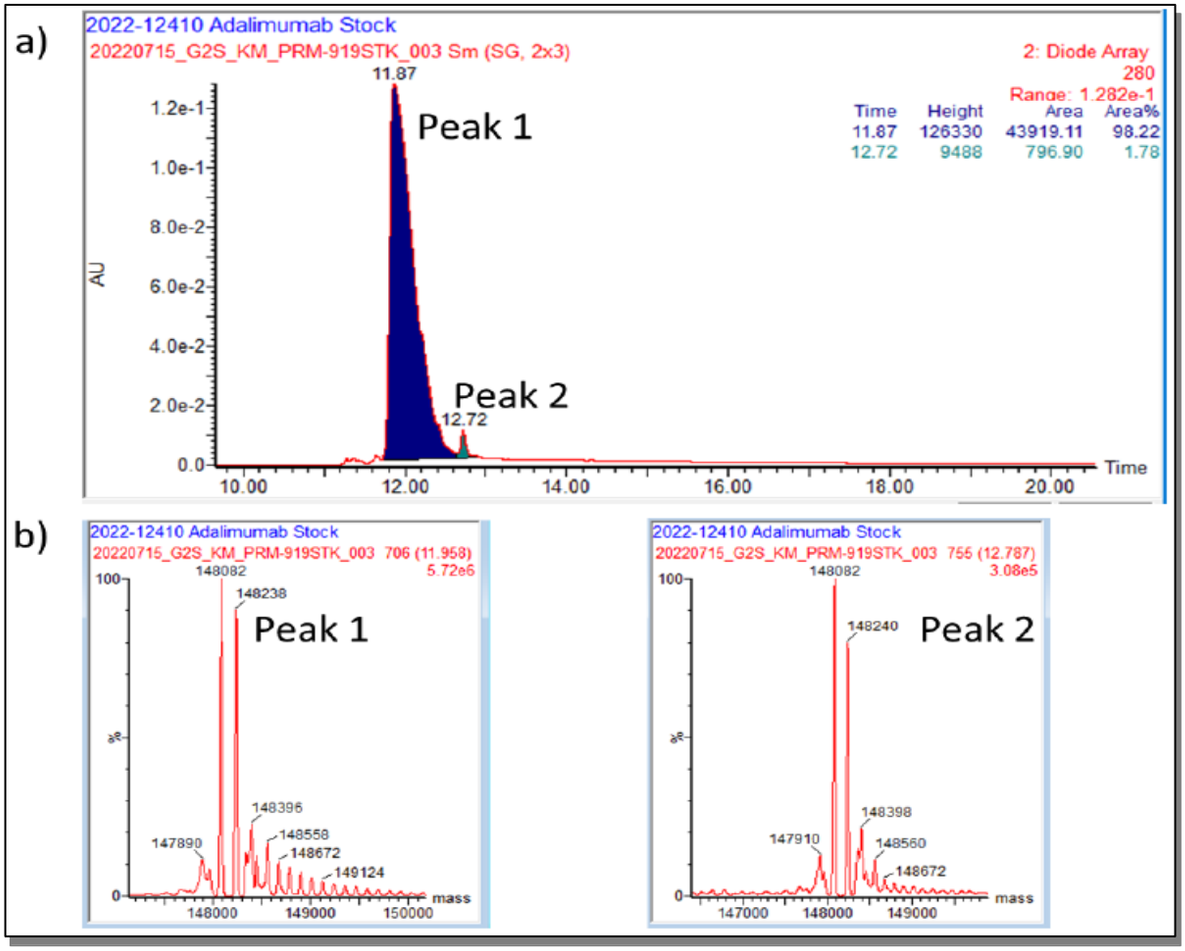
Column: BIOshell 400A Protein C4™, 3.4 µm, 150 x 2.1 mm

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

Gradient:

Time (min)	%A	%B
0.00	75	25
25.00	50	50
25.01	2	98
30.00	2	98
30.01	75	25
35.00	75	25

Flow Rate: 300 µL/min
Wavelength: 280 nm
Ionization: Electrospray, Positive Ion
Instrument: Waters Xevo G2S Q-ToF
Acquired: July 22, 2022



a) UV_{280} chromatogram
b) Deconvoluted mass spectra for peaks 1 and 2 for the intact Adalimumab peaks

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	

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

Long Term Stability: *Long term stability has been assessed for Freezer storage (-10 °C to -25 °C) conditions. Stability of a minimum of 30 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	October 14, 2022	Initial version
01	April 16, 2025	Revised Quality Assurance Manager signature
		Revised Retest Date of October 2024 to June 2025.