

Certified Reference Material

Reference material certificate

Stable Isotope Labeled Amino Acid Mix Solution 1

TraceCERT®
Traceable Certified Reference Materials

Product no.: 96378
Lot no.: BCCJ0804
Description of CRM: 17 stable isotope labelled Amino acids in 0.1 M hydrochloric acid
Expiry date: FEB 2026
Storage: Store at -20°C ± 5°C
Density at 20°C: 1002.1 kg m⁻³ ± 0.5 kg m⁻³ (k = 2)

Constituent	Certified values at 20°C and expanded uncertainties, $U = k \cdot u$ (k = 2) [1][2]	
Alanine- ¹³ C ₃ , ¹⁵ N	2.50 mmol/L ± 0.07 mmol/L	0.232 mg/g ± 0.006 mg/g
Arginine- ¹³ C ₆	2.51 mmol/L ± 0.07 mmol/L	0.451 mg/g ± 0.012 mg/g
Aspartic acid- ¹³ C ₄	2.50 mmol/L ± 0.07 mmol/L	0.342 mg/g ± 0.009 mg/g
Cystine- ¹³ C ₆ , ¹⁵ N ₂	1.25 mmol/L ± 0.03 mmol/L	0.309 mg/g ± 0.008 mg/g
Glutamic acid- ¹³ C ₅	2.50 mmol/L ± 0.07 mmol/L	0.380 mg/g ± 0.010 mg/g
Glycine- ¹³ C ₂ , ¹⁵ N	2.50 mmol/L ± 0.08 mmol/L	0.194 mg/g ± 0.006 mg/g
Histidine- ¹³ C ₆	2.50 mmol/L ± 0.07 mmol/L	0.402 mg/g ± 0.011 mg/g
Isoleucine- ¹³ C ₆ , ¹⁵ N	2.50 mmol/L ± 0.07 mmol/L	0.345 mg/g ± 0.010 mg/g
Leucine- ¹³ C ₆ , ¹⁵ N	2.50 mmol/L ± 0.07 mmol/L	0.344 mg/g ± 0.010 mg/g
Lysine- ¹³ C ₆	2.50 mmol/L ± 0.07 mmol/L	0.379 mg/g ± 0.011 mg/g
Methionine- ¹³ C ₅ , ¹⁵ N	2.50 mmol/L ± 0.07 mmol/L	0.387 mg/g ± 0.011 mg/g
Phenyl- ¹³ C ₆ -alanine	2.50 mmol/L ± 0.07 mmol/L	0.426 mg/g ± 0.012 mg/g
Proline- ¹³ C ₅	2.50 mmol/L ± 0.10 mmol/L	0.300 mg/g ± 0.012 mg/g
Serine- ¹³ C ₃ , ¹⁵ N	2.50 mmol/L ± 0.07 mmol/L	0.272 mg/g ± 0.008 mg/g
Threonine- ¹³ C ₄	2.50 mmol/L ± 0.07 mmol/L	0.307 mg/g ± 0.008 mg/g
Tyrosine-(phenyl- ¹³ C ₆)	2.50 mmol/L ± 0.07 mmol/L	0.467 mg/g ± 0.012 mg/g
Valine- ¹³ C ₅	2.50 mmol/L ± 0.09 mmol/L	0.305 mg/g ± 0.011 mg/g

Certificate issue date: May 25, 2023



ISO 17034
SRMS 0001

H. Sprecher

H.Sprecher – CRM Operations

Dr. P. Zell

Dr. P. Zell – Approving Officer



Metrological traceability:	Traceable to NIST SRM. Details see "Certification process details". ^[3]
Measurement method:	The certified value is established by weighing of well characterized starting materials in accordance with ISO/IEC 17025 ^[4]
Intended use:	This stable isotope labeled certified reference material (CRM) is suitable to be used as internal standard for quantitation of amino acid levels by LC/MS or GC/MS for isotope dilution methods and other research application.
Instructions for handling and correct use:	Shake well and allow to warm to room temperature before opening the ampule. Open a new ampule for each measurement series.
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.
Packaging:	Amber glass ampule
Accreditation:	Sigma-Aldrich Production GmbH is accredited by the Swiss Accreditation Service SAS as reference material producer under no. SRMS 0001 in accordance with international standard ISO 17034. ^[5]

Certification process details:

To guarantee top reliability of the values for this *TraceCERT*® certified reference material, well-established procedures were followed. The values have to agree in the range of their uncertainties, but the value from the gravimetric preparation has been chosen as certified value:

1. Gravimetric preparation using well-characterized materials is a practical realization of concentration units, through conversion of mass to amount of substance.^[6] If the purity of the materials is demonstrated and if contamination and loss of material is strictly prevented this approach allows highest accuracy. The certified value of *TraceCERT*® certified reference materials is based on this approach and directly traceable to the SI unit kilogram. Therefore, comprehensively characterized materials are used. All balances are certified by DKD and calibrated with OIML Class E2 (up to 12 kg) and F2 (up to 64 kg) weights.
2. The mass fraction of the main isotopologue is determined by LC-MS and is referred to the sum of all detectable ions of varying isotopic composition ([M-1], [M-2], etc.). Intensities of overlapping signals of isotopologues are corrected according to the natural abundance using suitable calculators, e.g. "enviPat".^[7] Mixed isotopic compositions, e.g. ¹³C and ¹⁵N are not resolved but contributions to the measurement uncertainty are included. This also refers to the molecular masses, which are used for the calculation of the mass fractions. Additional HRMS measurements confirm the identity of each isotopologue.
3. The starting material is measured by qNMR against a certified reference material which is traceable to the SI by the use of SRM® from NIST followed by gravimetric preparation using balances calibrated with SI-traceable weights. Consequently, the value calculated by this unbroken chain of comparisons is traceable to the reference to which the starting material is compared. Due to the nature of qNMR measurements, different analytes can be characterized using only two different SRM® from NIST (see table "Details on starting material" on page 3).
4. Samples of this mixture in the final packaged form are measured by LC-MS (IDMS) against a similar mixture of compounds with the same composition to underpin the gravimetric values.
5. Density measurement is performed in accordance with ISO/IEC 17025^[4]
6. The certificate is designed in accordance with ISO Guide 31.^[8]

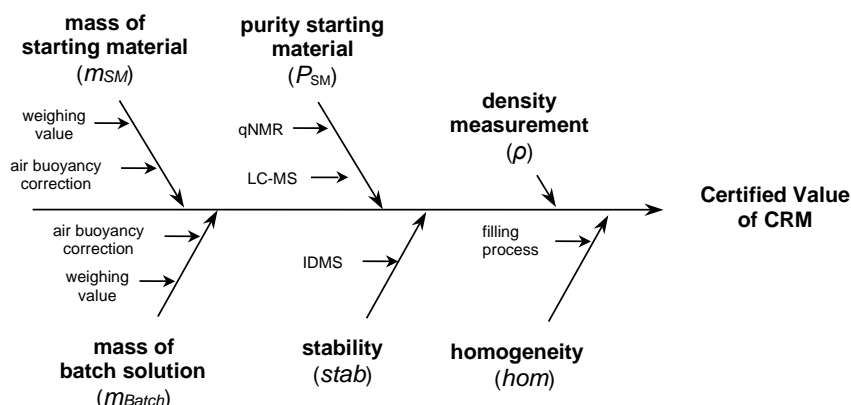
Homogeneity assessment:	Due to the nature of the production process, a homogeneous solution derives. Nevertheless, homogeneity is assessed by LC measurements and a homogeneity contribution is included into the calculation of content uncertainty of this CRM.
Stability assessment:	A stability study is performed with samples which are stored at different temperatures. The material is tested by LC-IDMS after certain time intervals and a stability component included in the overall uncertainty.

Uncertainty evaluation:

The uncertainty contributions are illustrated by the following cause-effect diagram:

Typical relative contributions are:

$u(P_{SM})$	< 1.7	%
$u(m_{SM})$	< 0.3	%
$u(m_{Batch})$	< 0.3	%
$u(\rho)$	< 0.1	%
$u(stab)$	< 1.0	%
$u(hom)$	< 0.2	%



The combined standard uncertainty is calculated by combination of the standard uncertainties of the input estimates according to Eurachem/CITAC Guide "Quantifying Uncertainty in Analytical Measurement" and ISO 17034. [2][5] Expanded uncertainty is then calculated to a confidence level of 95%, typically by multiplying with a coverage factor of $k=2$.

Details on starting materials:

Compound	CAS Number	Certified values (non stereo-specific) with expanded uncertainties ($k = 2$)	NIST SRM traceability
Glycine- $^{13}\text{C}_2, ^{15}\text{N}$	211057-02-2	97.9 % \pm 2.5 %	NIST SRM 84I (KHP)
L-Alanine- $^{13}\text{C}_3, ^{15}\text{N}$	202407-38-3	97.4 % \pm 2.6 %	NIST SRM 350b (Benzoic Acid)
L-Arginine- $^{13}\text{C}_6$	55443-58-8	76.9 % \pm 2.4 %	NIST SRM 84I (KHP) and NIST SRM 350b (Benzoic Acid)
L-Aspartic acid- $^{13}\text{C}_4$	55443-54-4	96.0 % \pm 2.6 %	NIST SRM 84I (KHP)
L-Cystine- $^{13}\text{C}_6, ^{15}\text{N}_2$	1252803-65-8	92.7 % \pm 2.5 %	NIST SRM 84I (KHP)
L-Glutamic acid- $^{13}\text{C}_5$	55443-55-5	94.8 % \pm 2.5 %	NIST SRM 84I (KHP)
L-Histidine- $^{13}\text{C}_6$	55443-59-9	71.9 % \pm 2.6 %	NIST SRM 84I (KHP) and NIST SRM 350b (Benzoic Acid)
L-Isoleucine- $^{13}\text{C}_6, ^{15}\text{N}$	202468-35-7	95.0 % \pm 2.6 %	NIST SRM 84I (KHP)
L-Leucine- $^{13}\text{C}_6, ^{15}\text{N}$	202406-52-8	95.4 % \pm 2.6 %	NIST SRM 84I (KHP)
L-Lysine- $^{13}\text{C}_6$	55443-57-7	73.1 % \pm 2.7 %	NIST SRM 84I (KHP)
L-Methionine- $^{13}\text{C}_5, ^{15}\text{N}$	202468-47-1	93.1 % \pm 2.8 %	NIST SRM 84I (KHP)
L-Phenyl- $^{13}\text{C}_6$ -alanine	180268-82-0	96.1 % \pm 2.8 %	NIST SRM 84I (KHP)
L-Proline- $^{13}\text{C}_5$	201740-83-2	95.6 % \pm 3.0 %	NIST SRM 84I (KHP)
L-Serine- $^{13}\text{C}_3, ^{15}\text{N}$	202407-34-9	97.2 % \pm 2.7 %	NIST SRM 84I (KHP)
L-Threonine- $^{13}\text{C}_4$	55443-53-3	91.7 % \pm 2.5 %	NIST SRM 84I (KHP)
L-Tyrosine-(phenyl- $^{13}\text{C}_6$)	201595-63-3	90.4 % \pm 2.5 %	NIST SRM 84I (KHP)
L-Valine- $^{13}\text{C}_5$	55443-52-2	92.7 % \pm 3.3 %	NIST SRM 84I (KHP)

References:

- [1] ISO Guide 35:2017, "Reference materials - Guidance for characterization and assessment of homogeneity and stability"
- [2] Eurachem/CITAC Guide, 3rd Ed. (2012), "Quantifying uncertainty in analytical measurement"
- [3] Eurachem/CITAC Guide, 2nd Ed. (2019), "Metrological traceability in chemical measurement" ISO
- [4] The accredited testing laboratory STS 0490 performs the measurements and weighing steps for the certification of this CRM under ISO/IEC 17025:2017, "General requirements for the competence of testing and calibration laboratories"
- [5] 17034:2016, "General requirements for the competence of reference material producers"
- [6] Reichmuth, A., Wunderli, S., Weber, M., Meyer, V. R. (2004), "The uncertainty of weighing data obtained with electronic analytical balances", Microchimica Acta 148: 133-141.
- [7] Loos, M., Gerber, C., Corona, F., Hollender, J., Singer, H. (2015). Accelerated isotope fine structure calculation using pruned transition trees, Analytical Chemistry 87(11), 5738-5744.
- [8] ISO Guide 31:2015, "Reference materials - Contents of certificates, labels and accompanying documentation"

Certificate of analysis revision history:

Certificate version	Date	Reason for version
01	May 25, 2023	Initial version

Disclaimer:

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