



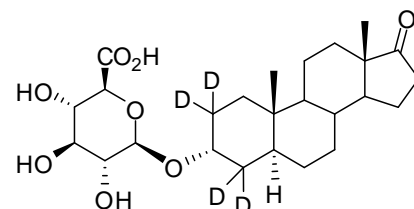
DEUTERATED INTERNAL STANDARD PRODUCT INFORMATION SHEET

NMIA S010: d4-5 α -Androstane-3 α ,17 β -diol-3-O- β -Glucuronic acid

Report ID: S010.2019.03

Chemical Formula: C₂₅H₃₆D₄O₈

Molecular Weight: 472.6 g/mol



Property value

Batch No.	CAS No.	Purity by HPLC-ELSD
11-S-07	Not Available	99.8 ± 0.02%

Expiration of certification: The property values are valid till 19 December 2029, i.e. ten years from the date of re-certification provided the **unopened** material is handled and stored in accordance with the recommendations below. The material as issued in the unopened container and stored as recommended below should be suitable for use beyond this date, subject to confirmation of batch stability from the issuing body. The expiry date/shelf life does not apply to sample bottles that have been opened. In such cases it is recommended that the end-user conduct their own in-house stability trials.

Description: White solid prepared by synthesis, and certified for identity and purity by NMIA. Packaged in amber glass bottles with a septum and crimped aluminium cap or screw top cap.

Intended use: The isotopic purity of this material is an estimate only. This material should be considered for use as an internal standard only.

Instructions for use: Equilibrate the bottled material to room temperature before opening.

Recommended storage: When not in use this material should be stored at or below 4 °C in a closed container in a dry, dark area.

Stability: The long-term stability of the compound in solution has not been examined.

Homogeneity assessment: The homogeneity of the material was assessed using purity assay by HPLC with ELS detection on ten randomly selected 1-2 mg sub samples of the material. The material was judged to be sufficiently homogeneous at this level of sampling as the variation in analysis results between samples was not significantly different at a 95% confidence level from that observed on repeat analysis of the same sample.

Safety: Treat as a hazardous substance. Use appropriate work practices when handling to avoid skin or eye contact, ingestion or inhalation of dust. Refer to the provided safety data sheet.



Dr Stephen R. Davies,
Team Leader,
Chemical Reference Materials, NMI.
16 November 2022

This report supersedes any issued prior to 16 November 2022.

NATA Accreditation No. 198 / Corporate Site No. 14214.

Legal notice: Terms and Conditions associated with the provision of this reference material can be found on the NMIA website.

Characterisation Report:

The identity was confirmed by a range of spectroscopic techniques, NMR, IR and MS. The purity estimate was obtained HPLC with ELS detection.

Supporting evidence is provided by elemental microanalysis.

The main component of this material is d4-5 α -androstane-3 α ,17 β -diol-3-O- β -glucuronic acid. d3-, d2-, d1- and d0-5 α -Androstane-3 α ,17 β -diol-3-O- β -glucuronic acid are also present. The stated chemical purity of the analyte represents the combined mass fractions of deuterated (d4, d3, d2 and d1) and d0-5 α -androstane-3 α ,17 β -diol-3-O- β -glucuronic acid in the material.

The isotopic purity of this material is an estimate only. This material should be considered for use as an internal standard only.

Isotopic Purity: $d_4 \approx 83\%$ [= $d_4/(d_4 + d_3 + d_2 + d_1 + d_0) \times 100$]

$d_0 < 0.2\%$ [= $d_0/(d_4 + d_3 + d_2 + d_1 + d_0) \times 100$]

[from SIM analysis of the *bis*-TMS derivatised free steroid]

HPLC: Instrument: Waters Model 1525 Binary pump, 717 plus autosampler
Column: X-Bridge C-18, 5 μ m (4.6 mm x 150 mm)
Column oven: 40 $^{\circ}$ C
Mobile Phase: Methanol/MilliQ water (60:40 v/v)
Formic acid (0.5% v/v) was present in the aqueous phase
Flow rate: 1.0 mL/min
Detector: Waters ELSD 2424
Relative peak area of the main component:
Initial analysis: Mean = 99.8%, s = 0.02% (10 sub samples in duplicate, September 2012)

Karl Fischer analysis: Moisture content 2.9% mass fraction (September 2012)
Moisture content 13% mass fraction (December 2019)

Thermogravimetric analysis: Volatiles content 1.7% and non-volatile residue < 0.2% mass fraction (September 2012)

Spectroscopic and other characterisation data

ESI-MS:	Instrument:	Micromass Quatro LC Micro
	Operation:	Negative ion mode, direct infusion at 5 μ L/min
	Ionisation:	ESI spray voltage at 3.0 kV negative ion
	EM voltage:	650 V
	Cone voltage:	20 V
	Peak:	471.5 (M-H ⁺) <i>m/z</i>
TLC:	Conditions:	Kieselgel 60F ₂₅₄ . Chloroform/methanol (2:1) Single spot observed, R _f = 0.9 Visualisation with vanillin
IR:	Instrument:	Biorad FTS3000MX FT-IR
	Range:	4000-400 cm ⁻¹ , KBr powder
	Peaks:	3420, 2917, 2868, 2203, 2109, 1716, 1446, 1266, 1218, 1161, 1056, 1016 cm ⁻¹
¹ H NMR:	Instrument:	Bruker Avance DMX-600
	Field strength:	600 MHz
	Solvent:	MeOH- <i>d</i> ₄ (3.31 ppm)
	Spectral data:	δ 0.72 (3H, s), 0.78 (1H, m), 0.83 (3H, s), 0.89-1.04 (3H, m), 1.14-1.32 (4H, m), 1.36 1.48 (4H, m), 1.55-1.61 (2H, m), 1.63-1.68 (2H, m), 1.81 (1H, ddd, <i>J</i> = 12.2, 3.1, 3.1 Hz), 1.96 (1H, m), 3.24 (1H, dd, <i>J</i> = 7.9, 9.3 Hz), 3.38 (1H, t, <i>J</i> = 9.1 Hz), 3.53 (1H, t, <i>J</i> = 9.6 Hz), 3.55 (1H, t, <i>J</i> = 8.7 Hz), 3.76 (1H, d, <i>J</i> = 9.8 Hz), 3.93, (1H, s), 4.37 (1H, d, <i>J</i> = 7.8 Hz) ppm
¹³ C NMR:	Instrument:	Bruker Avance III-500
	Field strength:	151 MHz
	Solvent:	MeOH- <i>d</i> ₄ (49.0 ppm)
	Spectral data:	δ 11.7, 11.9, 21.5, 24.3, 25.8, 29.5, 30.6, 32.9, 33.5, 34.5, 36.9, 38.1, 40.3, 44.1, 52.5, 55.8, 73.2, 74.8, 75.5, 76.6, 77.7, 82.6, 103.1, 172.7 ppm
Melting point:		225-228 °C
Microanalysis:	Found:	C = 62.1%; H = 8.7% (September 2012)
	Calculated:	C = 61.7%; H = 8.7% (Calculated for C ₂₅ H ₃₆ D ₄ O ₈ + 3% water)