



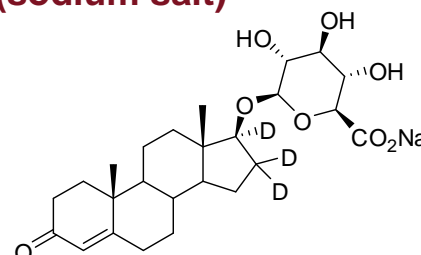
DEUTERATED INTERNAL STANDARD PRODUCT INFORMATION SHEET

NMIA S036: d₃-Testosterone-17-O-β-glucuronic acid (sodium salt)

Report ID: S036.2020.01

Chemical Formula: C₂₅H₃₂D₃O₈Na

Molecular Weight: 489.5 g/mol



Property value

Batch No.	CAS No.	Purity estimate
15-S-06	N/A	87.9%

Synonyms: Testosterone-1,16,16,17-d₃ glucuronide sodium salt
d₃-Testosterone glucuronoside sodium salt
d₃-Testosterone glucosiduronate sodium salt

Expiration of certification: The property values are valid till 14 August 2025, i.e. five 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 UV detection on seven 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.

S. R. Davies

Dr Stephen R. Davies,
Team Leader,
Chemical Reference Materials, NMI.
24 August 2020.

This report supersedes any issued prior to 24 August 2020.

NATA logo notice: Accredited for compliance with ISO Guide 17034. Accreditation No. 198 / Corporate Site No. 20844. The results of the tests, calibrations and/or measurements included in this document are traceable to Australian/national standards.

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 value was obtained by mass balance from a combination of traditional analytical techniques, including HPLC with UV detection, thermogravimetric analysis and ¹H NMR. The purity value is calculated as per Equation 1.

$$\text{Purity} = (100 \% - I_{\text{ORG}}) \times (100 \% - I_{\text{VOL}} - I_{\text{NVR}}) \quad \text{Equation 1}$$

I_{ORG} = Organic impurities of related structure, I_{VOL} = volatile impurities, I_{NVR} = non-volatile residue.

Supporting evidence is provided by elemental microanalysis.

The main component of this material is d₃-testosterone-17-O-β-glucuronic acid sodium salt. d₂-, d₁- and d₀-testosterone-17-O-β-glucuronic acid sodium salt are also present. The stated chemical purity of the analyte represents the combined mass fractions of deuterated (d₃, d₂ and d₁) and d₀-testosterone-17-O-β-glucuronic acid sodium salt 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: $d_3 \approx 96\% [= d_3 / (d_0 + d_1 + d_2 + d_3) \times 100]$
 $d_0 < 0.4\% [= d_0 / (d_0 + d_1 + d_2 + d_3) \times 100]$
[from SIM analysis of the *bis*-TMS derivative of the free steroid]

HPLC: Instrument: Waters Model 1525 Binary pump, 717 plus autosampler
Column: Alltima C-18, 5 μm (4.6 mm x 150 mm)
Column oven: 40 °C
Mobile Phase: Methanol/Milli-Q water (55:45)
The aqueous phase contained 0.1% formic acid
Flow rate: 1.0 mL/min
Detector: Waters PDA 2998 operating at 246 nm
Relative peak area of main component:
Initial analysis: Mean = 95.6%, s = 0.1% (7 sub samples in duplicate, August 2015)

HPLC: Instrument: Waters Alliance 2695 Separations Module
Column: Alltima C-18, 5 μm (4.6 mm x 150 mm)
Column oven: 40 °C
Mobile Phase: A = 0.1% formic acid in MilliQ water, B = Methanol
Gradient: 0-12 min 51% B, 12-14 min 51-80% B, 14-20 min 80% B, 20-21 min 80-51% B, 21-30 min 51% B
Flow rate: 1.0 mL/min
Detector: Waters PDA 2998 operating at 246 nm
Relative peak area of main component:
Initial analysis: Mean = 97.0%, s = 0.02% (5 sub samples in duplicate, August 2020)

Thermogravimetric analysis: Volatile content 7.2% (July 2015)

Karl Fischer analysis: Moisture content 9.6% mass fraction (July 2015)
Moisture content 9.2% mass fraction (June 2020)

Spectroscopic and other characterisation data

GC-MS:	The free steroid was liberated upon treatment with methanolic HCl, and derivatised with MSTFA. Instrument: Agilent 6890/5973 Column: HP-1MS, 30 m x 0.25 mm I.D. x 0.25 μm Program: 180 °C (1 min), 30 °C/min to 250 °C (10 min), 30 °C/min to 300 °C (2 min) Injector: 250 °C Transfer line temp: 280 °C Carrier: Helium, 1.0 mL/min Split ratio: 20/1 The retention time of the <i>bis</i> -TMS derivative of d ₃ -testosterone is reported along with the major peaks in the mass spectrum. The latter are reported as mass/charge ratios and (in brackets) as a percentage relative to the base peak. <i>Bis</i> -TMS (11.6 min): 435 (M ⁺ , 100), 420 (14), 209 (10), 73 (52) <i>m/z</i>
ESI-MS:	Instrument: Micromass Quatro LC Micro Operation: Negative ion mode, direct infusion at 10 μL/min Ionisation: ESI spray voltage at 3.5 kV positive ion EM voltage: 650 V Cone voltage: 50 V Peak: 466.4 (M-Na ⁺) ⁻ <i>m/z</i>
TLC:	Conditions: Kieselgel 60F254. Chloroform/methanol/water (70:30:2) Single spot observed, R _f = 0.12. Visualisation with UV at 254 nm
IR:	Instrument: Bruker Alpha FT-IR Range: 4000-400 cm ⁻¹ , neat Peaks: 3454 (br), 1667, 1613, 1417 cm ⁻¹ (This IR data is for the corresponding d ₃ -testosterone-17-O-β-glucuronic acid.)
¹ H NMR:	Instrument: Bruker Avance III 500 Field strength: 500 MHz Solvent: MeOH- <i>d</i> ₄ (3.31 ppm) Spectral data: δ 0.90 (3H, s), 0.94-1.06 (3H, m), 1.20-1.32 (2H, m), 1.24 (3H, s), 1.51 (1H, dddd, <i>J</i> = 4.0, 13.3, 13.3, 13.3 Hz), 1.56-1.66 (3H, m), 1.70 (1H, ddd, <i>J</i> = 4.2, 13.9, 13.9 Hz), 1.89 (1H, m), 2.02-2.10 (2H, m), 2.25-2.33 (2H, m), 2.44-2.51 (2H, m), 3.20 (1H, t, <i>J</i> = 8.5 Hz), 3.37 (1H, t, <i>J</i> = 8.7 Hz), 3.42 (1H, t, <i>J</i> = 9.5 Hz), 3.50 (1H, d, <i>J</i> = 9.6 Hz), 4.35 (1H, d, <i>J</i> = 7.7 Hz), 5.71 (1H, s) ppm
¹³ C NMR:	Instrument: Bruker Avance III 500 Field strength: 126 MHz Solvent: MeOH- <i>d</i> ₄ (49.0 ppm) Spectral data: δ 12.0, 17.7, 21.8, 23.9, 32.8, 33.9, 34.7, 36.7, 38.5, 40.0, 44.1, 51.7, 55.4, 73.8, 75.3, 76.3, 77.9, 104.4, 124.1, 175.3, 176.9, 202.4 ppm
Melting point:	> 250 °C (decomposition)
Microanalysis:	Found: C = 55.1%; H = 7.5% (August 2015) Calculated: C = 55.4%; H = 7.7% (Calculated for C ₂₅ H ₃₂ D ₃ O ₈ Na plus 9.6% water)