Australian Government
Department of Industry, Science and Resources

National Measurement Institute

NATA
WORLD RECOGNISED ACCREDITATION

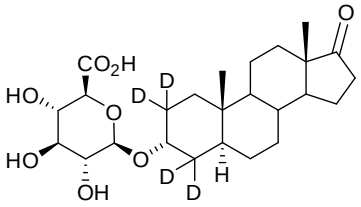
DEUTERATED INTERNAL STANDARD PRODUCT INFORMATION SHEET

NMIA D829h: d4-Androsterone-β-glucuronic acid

Report ID: D829h.2025.01 (Ampouled 240530)

Chemical Formula: $C_{25}H_{34}D_4O_8$

Molecular Weight: 470.5 g/mol



Property value

Batch No.	CAS No.	Mass per ampoule
23-S-05	**N/A**	**823 ± 40 µg**

The uncertainty has been calculated according to ISO Guide 35 and is stated at the 95% confidence limit (k = 2).

IUPAC name: (3α,5α)-17-Oxo(2,2,4,4-$^2H_4$) androstan-3-yl β-D-glucopyranosiduronic acid.

Expiration of certification: The property values are valid till 23 January 2028, three 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 shelf life does not apply to ampoules that have been opened. In such cases it is recommended that the end-user conduct their own in-house stability trials. The material will be re-tested on an annual basis to ensure that the property values are still valid. In the event a product fails the stability trial, notification will be sent to all impacted customers.

Description: The compound is supplied as a dried aliquot in a sealed ampoule under an atmosphere of argon. The deuterated internal standard is intended for a single use to prepare a standard solution containing D829h. The material was prepared by synthesis and certified for identity and purity by NMI Australia. The main component of this material is d_4-androsterone- β-glucuronic acid. d_3-, d_2-, d_1- and d_0- androsterone- β-glucuronic acid are also present. The stated mass of the analyte per ampoule represents the approximate combined masses of deuterated (d_4, d_3, d_2 and d_1) and d_0- androsterone- β-glucuronic acid in the material.

Intended use: The isotopic purity of this material is an estimate only. This material should be considered for use as an internal standard only and is not intended for use as a calibrator. The material does not have certified reference material status as metrological traceability of the stated purity value to the SI unit for mass (kg) has not been established.

Instructions for use: Open the ampoule and carefully rinse the interior at least three times with a suitable organic solvent (e.g. methanol). This will transfer approximately 823 ± 40 µg of anhydrous d_4-androsterone-β-glucuronic acid (d_3, d_2, d_1 and d_0). The mass of analyte in each ampoule is calculated from the assigned purity of the bulk and the concentration of bulk material in a stock solution used to prepare the ampoules.

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

Stability: In the absence of long-term stability data the measurement uncertainty at the 95% coverage interval has been expanded to accommodate any potential change in the property value. The stability component has been estimated from stability trials conducted on similar materials by NMI Australia over the last ten years.

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 charged aerosol detection on seven randomly selected ampoules 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.
14 May 2025.

This report supersedes any issued prior to 14 May 2025.

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

Characterisation Report:

HPLC:	Instrument:	Thermo Scientific Vanquish
	Column:	ACE Excel Super C-18, 5 μm (4.6 mm x 250 mm)
	Column oven:	35 °C
	Mobile Phase:	Acetonitrile/ 0.2% formic acid (38:62 v/v)
	Flow rate:	1.0 mL/min
	Detector:	Vanquish detector
	Summed relative peak area of d4-androsterone glucuronic acid and d4-epi-androsterone glucuronic acid:	
	Initial analysis:	Mean = 96.2%, s = 0.08% (7 ampoules in duplicate, June 2024)
	Re-analysis:	Mean = 96.3%, s = 0.04% (5 ampoules in duplicate, April 2025)

The main impurity in this material has been identified as d4-epi-androsterone glucuronic acid. The mole ratio of d4-androsterone glucuronic acid and d4-epi-androsterone glucuronic acid has been determined by ^{1}H NMR to be 90.5% and 9.5%, respectively.

The following analytical data was obtained on the bulk material subsequently used in the preparation of the ampoules.

The identity was confirmed by a range of spectroscopic techniques, NMR, IR and MS. The indicative purity value was obtained by mass balance from a combination of traditional analytical techniques, including HPLC with evaporative light scattering and charged aerosol detection, thermogravimetric analysis, Karl Fischer analysis, and ^{1}H NMR spectroscopy. The purity value is calculated as per Equation 1.

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

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 d_4-androsterone-β-glucuronic acid. d_3-, d_2-, d_1- and d_0-Androsterone-β-glucuronic acid are also present. The stated chemical purity of the analyte represents the combined mass fractions of deuterated (d_4, d_3, d_2 and d_1) and d_0-androsterone-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:	$d_4 \approx 89\%$ [= $d_4/(d_4 + d_3 + d_2 + d_1 + d_0) \times 100$]	
	$d_0 < 0.1\%$ [= $d_0/(d_4 + d_3 + d_2 + d_1 + d_0) \times 100$]	
HPLC:	Instrument:	Thermo Scientific Vanquish
	Column:	ACE Excel Super C-18, 5 μm (4.6 mm x 250 mm)
	Column oven:	35 °C
	Mobile Phase:	Acetonitrile/ 0.2% formic acid (38:62 v/v)
	Flow rate:	1.0 mL/min
	Detector:	Vanquish detector
	Summed relative peak area of d4-androsterone glucuronic acid and d4-epi-androsterone glucuronic acid:	
	Initial analysis:	Mean = 97.9%, s = 0.2% (10 sub samples in duplicate, January 2024)
HPLC:	Instrument:	Shimadzu Binary pump LC-20AB, SIL-20 A HT autosampler
	Column:	ACE Avantor C-18, 5 μm (4.6 mm x 150 mm)
	Column oven:	40 °C
	Mobile Phase:	Acetonitrile/ 0.2% formic acid (38:62 v/v)
	Flow rate:	1.0 mL/min
	Detector:	Shimadzu ELSD-LT II
	Summed relative peak area of d4-androsterone glucuronic acid and d4-epi-androsterone glucuronic acid:	
	Initial analysis:	Mean = 100%, s = 0.0% (10 sub samples in duplicate, January 2024)
Karl Fischer analysis:		Moisture content 8.5% mass fraction (January 2024)
Thermogravimetric analysis:		Volatiles content 9.8% and non-volatile residue < 0.2% mass fraction (January 2024)

Spectroscopic and other characterisation data

ESI-MS:	Instrument:	Shimadzu
	Operation:	Negative ion mode, direct infusion at 10 μL/min
	Interface voltage:	ESI spray voltage at 4.0 kV negative ion
	Peak:	469 ($M\text{-}H^-$) *m/z*
IR:	Instrument:	Bruker FTS3000MX FT-IR
	Range:	4000-400 cm^{-1}, KBr powder
	Peaks:	3396, 2919, 2857, 2361, 2342, 2197, 2106m 1734, 1088, 1054, 1017 cm^{-1}
^{1}H NMR:	Instrument:	Bruker Avance III-500
	Field strength:	500 MHz
	Solvent:	MeOH-d_4 (3.31 ppm)
	Spectral data:	δ 0.83 (1H, m), 0.85 (3H, s), 0.87 (3H, s), 1.03 (1H, m), 1.18-1.44 (7H, m), 1.50-1.85 (6H, m), 1.96 (1H, m), 2.06 (1H, td, *J* = 9.0, 19.5 Hz), 2.42 (1H, dd, *J* = 9.0, 19.5 Hz), 3.24 (1H, dd, *J* = 8.0, 9.0 Hz), 3.38 (3H, t, *J* = 9.0 Hz), 3.53 (1H, t, *J* = 9.0 Hz), 3.75 (1H, t, *J* = 9.5 Hz), 3.94 (1H, s), 4.37 (1H, d, *J* = 7.5 Hz) ppm
^{13}C NMR:	Instrument:	Bruker Avance III-500
	Field strength:	126 MHz
	Solvent:	MeOH-d_4 (49 ppm)
	Spectral data:	δ 11.9, 14.2, 21.2, 22.7, 29.3, 32.1, 32.9, 33.4, 36.4, 36.7, 37.0, 40.3, 49.1, 52.9, 55.7, 73.2, 74.8, 75.4, 76.6, 77.6, 103.0, 172.7, 224.2 ppm
Microanalysis:	Found:	C = 57.9%; H = 7.9% (February 2024)
	Calculated:	C = 63.8%; H = 8.2% (Calculated for $C_{25}H_{34}D_4O_8$) C = 59.3%; H = 9.2% (Calculated for $C_{25}H_{34}D_4O_8.2H_2O$, 7.1% water) C = 57.2%; H = 9.2% (Calculated for $C_{25}H_{34}D_4O_8.3H_2O$, 10.3% water)