Australian Government
Department of Industry, Science and Resources

National Measurement Institute

NATA
WORLD RECOGNISED ACCREDITATION

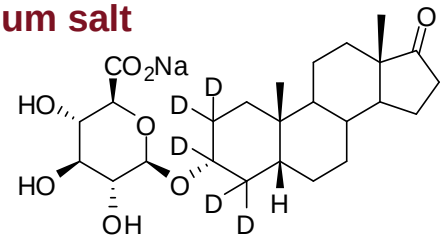
DEUTERATED INTERNAL STANDARD PRODUCT INFORMATION SHEET

NMIA S020b: d5-Etiocholanolone glucuronide sodium salt

Report ID: S020b.2025.01 (Ampouled 250501)

Chemical Formula: $C_{25}H_{32}D_5NaO_8$

Molecular Weight: 493.6 g/mol



Property value

Batch No.	CAS No.	Mass per ampoule
24-S-06	Not available	881 ± 23 µg

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

IUPAC name: Sodium (3α,5β)-17-oxo(2,2,3,4,4-$^2H_5$) androstan-3-yl β-D-glucopyranosiduronate.

Expiration of certification: The property values are valid till 8 May 2028, three years from the date of 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: White solid prepared by synthesis and certified for identity and purity by NMI Australia. The analyte is supplied as a dried aliquot in a sealed ampoule under an atmosphere of argon, intended for single use to prepare a standard solution. The main component of this material is d_5-etiocholanolone-β-glucuronide sodium salt. d_4-, d_3-, d_2-, d_1- and d_0-Etiocholanolone-β-glucuronide are also present. The stated mass of the analyte per ampoule represents the approximate combined masses of deuterated (d_5, d_4, d_3, d_2 and d_1) and d_0-etiocholanolone-β-glucuronide sodium salt 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 881 ± 23 µg of anhydrous d_5-etiocholanolone glucuronide sodium salt. 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: At the recommended storage conditions this material has demonstrated stability for a period of three 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 eight 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.
22 May 2025.

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:

HPLC:	Instrument:	Thermo Scientific Vanquish
	Column:	ACE Excel 5 Super C18, 150 mm × 4.6 mm I.D. × 5 μm
	Column oven:	40°C
	Mobile Phase:	Methanol / MilliQ H_2O with 0.5% formic acid (65:35 v/v)
	Flow rate:	1.0 mL/min
	Detector:	Vanquish detector
	Relative peak area of the main component:	
	Initial analysis:	Mean = 99.4%, s = 0.03% (7 ampoules in duplicate, May 2025)

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 evaporative light scattering and charged aerosol detection, Karl Fischer analysis, and ^{1}H NMR spectroscopy. The purity value is calculated as per Equation 1.

Purity = (100 % - I_{ORG}) x (100 % - I_{VOL} – I_{NVR}) 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_5-etiocholanolone-β-glucuronide. d_4-, d_3-, d_2-, d_1- and d_0- etiocholanolone-β-glucuronide are also present. The stated chemical purity of the analyte represents the combined mass fractions of deuterated (d_5, d_4, d_3, d_2 and d_1) and d_0-etiocholanolone-β-glucuronide 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_5 ≈ 90% [= $d_5/(d_5 + d_4 + d_3 + d_2 + d_1 + d_0)$ x 100]	
	d_0 < 0.2% [= $d_0/(d_5 + d_4 + d_3 + d_2 + d_1 + d_0)$ x 100]	
HPLC:	Instrument:	Thermo Scientific Vanquish
	Column:	ACE Excel 5 Super C18, 150 mm × 4.6 mm I.D. × 5 μm
	Column oven:	40°C
	Mobile Phase:	Methanol / MilliQ H_2O with 0.5% formic acid (65:35 v/v)
	Flow rate:	1.0 mL/min
	Detector:	Vanquish detector
	Relative peak area of the main component:	
	Initial analysis:	Mean = 99.6%, s = 0.09% (10 sub samples in duplicate, August 2024)
HPLC:	Instrument:	Shimadzu Binary pump LC-20AB, SIL-20 A HT autosampler
	Column:	ACE Excel 5 Super C18, 150 mm × 4.6 mm I.D. × 5 μm
	Column oven:	40 °C
	Mobile Phase:	Methanol / MilliQ H_2O with 0.5% formic acid (65:35 v/v)
	Flow rate:	1.0 mL/min
	Detector:	Shimadzu ELSD-LT II
	Relative peak area of the main component:	
	Initial analysis:	Mean = 100.0%, s = 0.00% (10 sub samples in duplicate, August 2024)
Karl Fischer analysis:		Moisture content 10.7% mass fraction (August 2024)

Spectroscopic and other characterisation data

ESI-MS:	Instrument:	Shimadzu LC-TQ-MS 8045
	Operation:	Direct infusion at 10 μL/min
	Ionisation mode:	Electrospray negative ion
	Interface voltage:	4.0 kV
	Peak:	470 $(M\text{-}Na^{+})^{-}$ *m/z*
IR:	Instrument:	Bruker Alpha Platinum ATR
	Range:	4000-400 cm^{-1}, neat
	Peaks:	3615, 3520, 3454, 2934, 2923, 2866, 2853, 2200, 2130, 1730, 1612, 1408, 1376, 1295, 1167, 1087, 1070, 1042, 1027 cm^{-1}
^{1}H NMR:	Instrument:	Bruker Avance III-500
	Field strength:	500 MHz
	Solvent:	MeOH-d_4 (3.31 ppm)
	Spectral data:	δ 0.87 (3H, s), 0.98 (3H, s), 0.99 (1H, d, *J* =14.5 Hz), 1.21-1.45 (6H, m), 1.51-1.69 (5H, m), 1.76 (1H, m), 1.82 (1H, d, *J* = 14.0 Hz),1.89-1.99 (2H, m), 2.06 (1H, ddd, *J* = 9.0, 9.0, 18.5 Hz), 2.43 (1H, dd, *J* = 8.5, 19.0 Hz), 3.18 (1H, t, *J* = 8.0 Hz), 3.39 (1H, t, *J* = 8.5 Hz), 3.43 (1H, t, *J* = 9.0 Hz), 3.54 (1H, d, *J* = 9.5 Hz), 4.40 (1H, d, *J* = 8.0 Hz), ppm
^{13}C NMR:	Instrument:	Bruker Avance III-500
	Field strength:	126 MHz
	Solvent:	MeOH-d_4 (49 ppm)
	Spectral data:	δ 14.2, 21.2, 22.8, 23.8, 26.5, 28.0, 33.0, 35.9, 36.1, 36.7, 36.8, 42.1, 43.4, 52.8, 73.7, 75.0, 76.1, 77.9, 101.8, 177.0, 224.2 ppm
Microanalysis:	Found:	C = 53.6%; H = 7.6% (August 2024)
	Calculated:	C = 60.8%; H = 7.7% (Calculated for $C_{25}H_{32}D_5O_8Na$)
	Calculated:	C = 54.8%; H = 8.0% (Calculated for $C_{25}H_{32}D_5O_8Na$ + 10% H_2O)