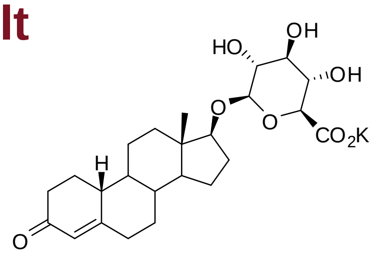
Australian Government
Department of Industry, Science and Resources

National Measurement Institute

NATA
WORLD RECOGNISED ACCREDITATION

CERTIFIED REFERENCE MATERIAL CERTIFICATE OF ANALYSIS

NMIA D861: Nandrolone glucuronic acid K salt



Report ID: D861.2022.01 (Ampouled 100805)

Chemical Formula: $C_{24}H_{33}O_8K$

Molecular Weight: 488.6 g/mol

Certified value

Batch No.	CAS No.	Mass per ampoule
03-S-16	**Not Available**	**897 ± 30 µg**

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

IUPAC: Potassium (17β)-17-Hydroxyestr-4-en-3-one β-D-glucopyranosiduronate

Expiration of certification: The property values are valid till 11 May 2027, 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 ampoules that have been opened. In such cases it is recommended that the end-user conduct their own in-house stability trials.

Description: The compound is supplied as a dried aliquot in a sealed ampoule under an atmosphere of argon. The CRM is intended for a single use to prepare a standard solution containing D861. This material was sourced from an external supplier, and certified for identity and purity by NMIA.

Intended use: This certified reference material is suitable for use as a primary calibrator.

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 897 ± 30 µg of anhydrous nandrolone glucuronic acid K 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.

Metrological traceability: The certified purity value is traceable to the SI unit for mass (kg) through Australian national standards via balance calibration. Quantitative NMR provides an independent direct measure of the mass fraction of the analyte of interest, calibrated with an internal standard certified for purity (mass fraction).

Stability: This material has demonstrated stability over a minimum period of five years. The measurement uncertainty at the 95% confidence interval includes a stability component which has been estimated from annual stability trials. 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 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 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.
28 July 2022

This report supersedes any issued prior to 28 July 2022.

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

Characterisation Report:

HPLC:	Instrument:	Waters Model 1525 Binary pump, 717 plus autosampler or Shimadzu Binary pump LC-20AB, SIL-20 A HT autosamplerThermo Scientific UltiMate 3000
	Column:	Grace Alltima C-18, 5µm (4.6 mm × 150 mm)
	Mobile Phase:	Acetonitrile/20 mM NH_4OAc buffer, pH 4.2 (22:78)
	Flow rate:	1 mL/min
	Detector:	Waters PDA 2998 or Shimadzu SPD-M20A PDA operating at 247 nm
	Relative mass fraction of the main component:	
	Initial analysis:	Mean = 99.7%, s = 0.004% (7 ampoules in duplicate, August 2010)
	Re-analysis:	Mean = 99.7%, s = 0.01% (5 ampoules in duplicate, September 2011)
	Re-analysis:	Mean = 99.7%, s = 0.02% (5 ampoules in duplicate, September 2012)
	Re-analysis:	Mean = 99.7%, s = 0.02% (5 ampoules in duplicate, September 2017)
	Re-analysis:	Mean = 99.7%, s = 0.01% (5 ampoules in duplicate, May 2022)

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 certified purity value by qNMR was obtained using the one-proton singlet at 5.7 ppm measured against a certified internal standard of maleic acid.

Supporting evidence is provided by mass balance from a combination of traditional analytical techniques, including HPLC with UV detection, Karl Fischer analysis and ^{1}H NMR spectroscopy and elemental microanalysis.

QNMR:	Instrument:	Bruker Avance-400
	Field strength:	400 MHz
	Solvent:	MeOH-d_4 (3.31 ppm)
	Internal standard:	Maleic acid (98.7% mass fraction)
	Initial analysis:	Mean = 86.7%, s = 0.7% (3 sub samples, March 2010)
HPLC:	Instrument:	Waters Model 600E pump, 717 plus autosampler
	Column:	Alltech Alltima C-18, 5µm (4.6 mm × 150 mm)
	Mobile Phase:	Acetonitrile/20mM NH_4OAc buffer, pH 4.2 (22:78)
	Flow rate:	1 mL/min
	Detector:	Waters PDA 996 operating at 247 nm
	Relative mass fraction of the main component:	
	Initial analysis:	Mean = 99.8%, s = 0.01% (5 sub samples in duplicate, January 2004)
	Re-analysis:	Mean = 99.7%, s = 0.02% (5 sub samples in duplicate, December 2007)
Karl Fischer analysis:		Moisture content 7% mass fraction (November 2007 and January 2009)

Spectroscopic and other characterisation data

ESI-MS:	Instrument:	Micromass Quatro LC Micro
	Operation:	Negative ion mode, direct infusion at 5 μL/min
	Ionisation:	ESI spray voltage at 3.0 kV negative ion
	EM voltage:	650 V
	Cone voltage:	40 V
	Peak:	449.3 (M-K$^+$) *m/z*
TLC:	Conditions:	Kieselgel 60F$_{254}$. Ethyl acetate/methanol/acetic acid (67:30:3) Single spot observed, R$_f$ = 0.3 Visualization with vanillin
IR:	Instrument:	BioRad FTS3000MX FT-IR
	Range:	4000-400 cm^{-1}, KBr powder
	Peaks:	3285, 2915, 2862, 1666, 1605, 1410, 1056 cm^{-1}
^{1}H NMR:	Instrument:	Bruker DMX-500
	Field strength:	500 MHz
	Solvent:	D_2O (4.79 ppm)
	Key spectral data:	δ 0.86 (3H, s), 1.92 (1H, bd), 2.04 (1H, m), 2.50 (1H, bd), 3.26 (1H, m), 3.47 (2H, m), 3.65 (1H, m), 3.83 (1H, t), 4.50 (1H, d), 5.87 (1H, s) ppm ^{1}H NMR indicates the presence of methanol (ca 0.2% mass fraction)
^{13}C NMR:	Instrument:	Bruker DMX-300
	Field strength:	75 MHz
	Solvent:	D_2O
	Spectral data:	δ 11.5, 23.0, 26.1, 26.3, 28.3, 31.0, 35.8, 36.0, 37.5, 40.0, 42.6, 43.3, 49.3, 49.7, 49.9, 72.4, 73.7, 76.1, 76.8, 89.4, 102.9, 123.4, 174.1, 176.1, 205.5 ppm
Melting point:		275 °C
Microanalysis:	Found:	C = 55.1%; H = 6.8% (December, 2008)
	Calculated:	C = 54.9%; H = 7.1% (Calculated for $C_{24}H_{33}O_8K.2H_2O$)