



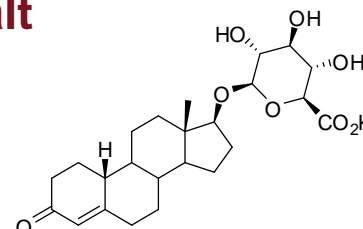
# CERTIFIED REFERENCE MATERIAL CERTIFICATE OF ANALYSIS

## NMIA D861: Nandrolone glucuronic acid K salt

Report ID: D861.2017.04 (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 5 September 2022, 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 °C 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 three 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.  
31 January 2020

This report supersedes any issued prior to 31 January 2020

**NATA logo notice:** Accredited for compliance with ISO 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.

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### Characterisation Report:

HPLC: Instrument: Waters Model 1525 Binary pump, 717 plus autosampler  
Column: Grace Alltima C-18, 5 $\mu$ m (4.6 mm  $\times$  150 mm)  
Mobile Phase: Acetonitrile/20 mM NH<sub>4</sub>OAc buffer, pH 4.2 (22:78)  
Flow rate: 1 mL/min  
Detector: Waters PDA 2998 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)

### 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 <sup>1</sup>H NMR spectroscopy and elemental microanalysis.

QNMNMR: Instrument: Bruker Avance-400  
Field strength: 400 MHz  
Solvent: MeOH-*d*<sub>4</sub> (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, 5 $\mu$ m (4.6 mm  $\times$  150 mm)  
Mobile Phase: Acetonitrile/20mM NH<sub>4</sub>OAc 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 $\mu$ L/min
	Ionisation:	ESI spray voltage at 3.0 kV negative ion
	EM voltage:	650 V
	Cone voltage:	40 V
	Peak:	449.3 (M-K <sup>+</sup> ) <i>m/z</i>
TLC:	Conditions:	Kieselgel 60F <sub>254</sub> . Ethyl acetate/methanol/acetic acid (67:30:3) Single spot observed, R <sub>f</sub> = 0.26 Visualization with vanillin
IR:	Instrument:	BioRad FTS3000MX FT-IR
	Range:	4000-400 $\text{cm}^{-1}$ , KBr powder
	Peaks:	3285, 2915, 2862, 1666, 1605, 1410, 1056 $\text{cm}^{-1}$
<sup>1</sup> H NMR:	Instrument:	Bruker DMX-500
	Field strength:	500 MHz
	Solvent:	D <sub>2</sub> O (4.79 ppm)
	Key spectral data:	$\delta$ 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 <sup>1</sup> H NMR indicates the presence of methanol (ca 0.2% mass fraction)
<sup>13</sup> C NMR:	Instrument:	Bruker DMX-300
	Field strength:	75 MHz
	Solvent:	D <sub>2</sub> O
	Spectral data:	$\delta$ 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 <sub>24</sub> H <sub>33</sub> O <sub>8</sub> K.2H <sub>2</sub> O)