Australian Government
Department of Industry, Science and Resources

National Measurement Institute

CIPM MRA

NATA
WORLD RECOGNISED ACCREDITATION

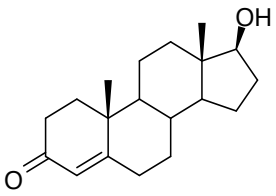
CERTIFIED REFERENCE MATERIAL CERTIFICATE OF ANALYSIS

NMIA M914c: Testosterone

Report ID: M914c.2025.01 (Bottled 190618)

Chemical Formula: $C_{19}H_{28}O_2$

Molecular Weight: 288.4 g/mol



Certified value

Batch No.	CAS No.	Purity (mass fraction)
10-S-01	**58-22-0**	**96.2 ± 1.5%**

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

Synonyms: 17β-Hydroxyandrost-4-en-3-one, Δ^4-Androsten-17β-ol-3-one, Androst-4-ene-17β-ol-3-one

Expiration of certification: The property values are valid till 8 April 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 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 powder sourced from an external supplier and certified for identity and purity by NMI Australia. Packaged in amber glass bottles with a septum and crimped aluminium cap.

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

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 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. In the mass balance all impurities are quantified as a mass fraction and subtracted from 100%. 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% coverage interval includes a stability component which has been estimated from long term 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 GC-FID on ten 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.

S. R. Davies

Dr Stephen R. Davies,
Team Leader,
Chemical Reference Materials, NMI.
11 June 2025

This report supersedes any issued prior to 11 June 2025.

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

CIPM MRA notice: This certificate is consistent with the capabilities that are included in Appendix C of the MRA drawn up by the CIPM. Under the CIPM MRA, all participating institutes recognise the validity of each other's calibration and measurement certificates for the quantities, ranges and measurement uncertainties specified in the KCDB (for details see http://www.bipm.org/kcdb/). The "CIPM MRA Logo" and this statement attest only to the measurement(s) applied for determining the certified values on the certificate.

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 certified purity value was obtained by mass balance from a combination of traditional analytical techniques, including GC-FID, thermogravimetric analysis, 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

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

Supporting evidence is provided by qualitative headspace GC-MS analysis of occluded solvents and elemental microanalysis.

GC-FID:	Instrument:	Varian CP-3800, Agilent 6890 and 8890
	Column:	HP-1 or HP-5, 30 m × 0.32 mm I.D. × 0.25 μm
	Program:	230 °C (16 min), 30 °C/min to 300 °C (3 min)
	Injector:	250 °C
	Detector Temp:	320 °C
	Carrier:	Helium
	Split ratio:	20/1
	Relative mass fraction of the main component:	
	Initial analysis:	Mean = 99.3%, s = 0.02% (10 sub samples in duplicate, May 2010)
	Re-analysis:	Mean = 99.3%, s = 0.01% (5 sub samples in duplicate, April 2016)
	Re-analysis:	Mean = 99.4%, s = 0.01% (10 sub samples in duplicate, August 2018)
	Re-analysis:	Mean = 99.4%, s = 0.03% (5 sub samples in duplicate, November 2021)
	Re-analysis:	Mean = 99.4%, s = 0.02% (5 sub samples in duplicate, February 2023)
	Re-analysis:	Mean = 99.5%, s = 0.01% (5 sub samples in duplicate, April 2025)
HPLC:	Instrument:	Thermofischer Dionex Ultimate 3000, RS pump, RS auto sampler
	Column:	Alltima C-18, 5.0 μm (4.6 mm x 150 mm)
	Column oven:	40 °C
	Mobile Phase:	Acetonitrile/MilliQ water (45:55 v/v)
	Flow rate:	1.0 mL/min
	Detector:	Dionex RS DAD operating at 245 nm
	Relative peak area of the main component:	
	Initial analysis:	Mean = 99.7%, s = 0.01% (7 sub samples in duplicate, May 2016)
QNMR:	Instrument:	Bruker Avance-III-500
	Field strength:	500 MHz
	Solvent:	DMSO-d_6 (2.50 ppm)
	Internal standard:	Dimethyl terephthalate (100.0% mass fraction)
	Initial analysis:	Mean (5.6 ppm) = 98.5%, s = 0.6% (8 sub samples, July 2016)
Thermogravimetric analysis:		Volatile content < 0.1 % and non-volatile residue < 0.2 % mass fraction (July 2011)
Karl Fischer analysis:		Moisture content < 0.2% mass fraction (May 2010) Moisture content 0.8% mass fraction (April 2016) Moisture content 1.3% mass fraction (July 2018) Moisture content 1.3% mass fraction (May 2019) Moisture content 3.3% mass fraction (November 2021) Moisture content 2.6% mass fraction (February 2023) Moisture content 3.3% mass fraction (April 2025)

Spectroscopic and other characterisation data

GC-MS:	Parent compound:	
	Instrument:	Agilent 6890/5973
	Column:	TG-1MS, 30 m x 0.25 mm I.D. x 0.25 μm
	Program:	180 °C (1 min), 10 °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
	Bis-TMS derivative:	
	Instrument:	Agilent 6890/5973
	Column:	TG-1MS, 30 m x 0.25 mm I.D. x 0.25 μm
	Program:	180 °C (1 min), 10 °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 times of the parent compound and *bis*-TMS derivative are reported with the major peaks in the mass spectra. The latter are reported as mass/charge ratios and (in brackets) as a percentage relative to the base peak.	
	Parent (11.21 min):	288 (M^+, 53), 246 (47), 228 (16), 203 (29), 187 (10), 185 (10), 159 (10), 147 (38), 124 (100), 105 (29), 91 (37), 79 (31), 67 (19), 55 (20), 41 (16) *m/z*
	Bis-TMS (11.9 min):	432 (M^+, 100), 417 (13), 301 (3), 209 (7), 73 (27) *m/z*
	The parent compound co-elutes with a comparison sample of testosterone.	
HS-GC-MS:	Instrument:	Agilent 6890/5973/G1888
	Column:	DB-624, 30 m x 0.25 mm I.D. x 1.4 μm
	Program:	50 °C (5 min), 7 °C/min to 120 °C, 15 °C/min to 220 °C (8.3 min)
	Injector:	150 °C
	Transfer line temp:	280 °C
	Carrier:	Helium, 1.2 mL/min
	Split ratio:	50/1
	Solvents detected:	Acetone
TLC:	Conditions:	Kieselgel 60F_{254}. Chloroform / ethyl acetate (4/1) Single spot observed, R_f = 0.3. Visualisation with UV at 254 nm
IR:	Instrument:	Biorad FTS300MX FT-IR
	Range:	4000-400 cm^{-1}, KBr powder
	Peaks:	3529, 3384, 2944, 2878, 1656, 1412, 1359, 1335, 1277, 1233, 1066, 1056, 1018, 956, 943, 870, 650, 513 cm^{-1}
^{1}H NMR:	Instrument:	Avance-DMX-600
	Field strength:	600 MHz
	Solvent:	$CDCl_3$ (7.26 ppm)
	Spectral data:	δ 0.77 (3H, s), 0.88-1.02 (3H, m), 1.06 (1H, ddd, *J* = 3.6, 12.8, 12.8 Hz), 1.17 (3H, s), 1.29 (1H, m), 1.37-1.48 (2H, m), 1.52-1.62 (3H, m), 1.67 (1H, ddd, *J* = 4.3, 14.1, 14.1), 1.75 (1H, bs), 1.83 (2H, m), 1.98-2.08 (2H, m), 2.24-2.43 (4H, m), 3.62 (1H, t, *J* = 8.6 Hz), 5.70 (1H, s) ppm
^{13}C NMR:	Instrument:	Avance-DMX-600
	Field strength:	151 MHz
	Solvent:	$CDCl_3$ (77.0 ppm)
	Spectral data:	δ 11.0, 17.4, 20.6, 23.3, 30.3, 31.5, 32.7, 33.9, 35.6, 35.7, 36.4, 38.6, 42.8, 50.4, 53.9, 81.5, 123.8, 171.3, 199.5 ppm
Melting point:		153-155 °C
Microanalysis:	Found:	C = 79.2%; H = 10.1% (May 2010)
	Calculated:	C = 79.1%; H = 9.8% (Calculated for $C_{19}H_{28}O_2$)