Australian Government
Department of Industry, Science and Resources

National Measurement Institute

NATA
WORLD RECOGNISED ACCREDITATION

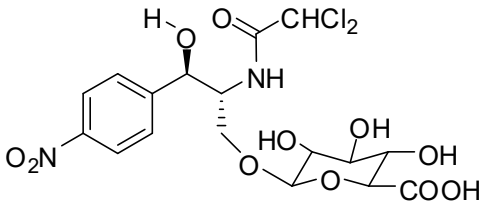
CERTIFIED REFERENCE MATERIAL CERTIFICATE OF ANALYSIS

NMIA P1808: Chloramphenicol glucuronic acid

Report ID: P1808.2025.03 (Ampouled 200526)

Chemical Formula: $C_{17}H_{20}N_2O_{11}Cl_2$

Molecular Weight: 499.3 g/mol



Certified value

Batch No.	CAS No.	Mass per ampoule
20-AV-01	39751-33-2	858 ± 17 µg

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

IUPAC name: (2*R*,3*R*)-2-[(Dichloroacetyl)amino]-3-hydroxy-3-(4-nitrophenyl)propyl β-D-glucopyranosiduronic acid.

Expiration of certification: The property values are valid till 23 April 2030, 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 sample bottles 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 P1808. This material was prepared by synthesis and certified for identity and purity by NMI Australia.

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 858 ± 17 µg of anhydrous chloramphenicol glucuronic acid. 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: 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.

Chloramphenicol glucuronic acid has been shown to be unstable in aqueous solution over prolonged periods, and monitoring of solutions over time is recommended. The long-term stability of the compound in a range of other solvents 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.
17 March 2026

This report supersedes any issued prior to 17 March 2026.

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 UltiMate 3000 or Waters alliance 2695 or Shimadzu HPLC
	Column:	Alltima C-18, 5 μm (4.6 mm × 150 mm), Ace Super C-18, 5 μm (4.6 mm × 250 mm)
	Column oven:	40 °C
	Mobile Phase:	A = Milli-Q water; B = Acetonitrile 0-13 min 30% B; 13-17 min 30-85% B; 17-23 min 85%B; 23-24 min 85-30% B, 24-30 min 30%B. The mobile phase contained 0.05% formic acid.
	Flow rate:	0.8 mL/min
	Detector:	Thermo Scientific Dionex UltiMate 3000 RS Diode Array operating or Waters 2998 PDA or Shimadzu PDA at 276 nm
	Relative mass fraction of the main component:	
	Initial analysis:	Mean = 98.6%, s = 0.017% (5 ampoules in duplicate, October 2023)
	Re analysis:	Mean = 98.8%, s = 0.006% (5 ampoules in duplicate, June 2025)
	Mobile Phase:	A = Milli-Q water with 5 mM NH_4OAc; B = Methanol 0-13 min 30% B; 13-17 min 30-85% B; 17-23 min 85%B; 23-24 min 85-30% B, 24-30 min 30%B. The aqueous phase was buffered at pH 4.2 using AcOH solution
	Flow rate:	0.8 mL/min
	Detector:	Thermo Scientific Dionex UltiMate 3000 RS Diode Array operating or Waters 2998 PDA or Shimadzu PDA at 276 nm
	Relative mass fraction of the main component:	
	Initial analysis:	Mean = 99.3%, s = 0.01% (7 ampoules in duplicate, June 2020)
	Re analysis:	Mean = 99.3%, s = 0.01% (5 ampoules in duplicate, June 2021)
	Re analysis:	Mean = 99.4%, s = 0.02% (5 ampoules in duplicate, April 2022)
	Re analysis:	Mean = 99.0%, s = 0.07% (5 ampoules in duplicate, March 2023)

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 two-proton doublet at 8.26 ppm measured against a certified internal standard of dimethyl sulfone.

Supporting evidence is provided by HPLC-UV analysis and ^1H NMR analysis.

QNMR:	Instrument:	Bruker Avance-III-500
	Field strength:	500 MHz
	Solvent:	D_2O (4.79 ppm)
	Internal standard:	Dimethyl sulfone (100.0% mass fraction)
	Initial analysis:	Mean (8.26 ppm) = 88.6%, s = 0.1% (5 sub samples, January 2020)
HPLC:	Instrument:	Thermo Scientific UltiMate 3000
	Column:	Alltima C-18, 5 μm (4.6 mm × 150 mm)
	Column oven:	40 °C
	Mobile Phase:	A = Milli-Q water; B = Methanol 0-13 min 30% B; 13-17 min 30-85% B; 17-23 min 85%B; 23-24 min 85-30% B, 24-30 min 30%B. The aqueous phase contained 5 mM NH_4OAc and was buffered at pH 4.2 using AcOH solution
	Flow rate:	0.8 mL/min
	Detector:	Thermo Scientific Dionex UltiMate 3000 RS Diode Array operating at 276 nm
	Relative mass fraction of the main component:	
	Initial analysis:	Mean = 99.0%, s = 0.03% (10 sub samples in duplicate, January 2020)
Karl Fischer analysis:		Moisture content 1.8% mass fraction (January 2020) Moisture content 2.3% mass fraction (June 2020)

Spectroscopic and other characterisation data

ESI-MS:	Instrument:	Micromass Quatro LC Micro
	Operation:	Negative ion mode, direct infusion at 20 μL/min
	Ionisation:	ESI spray voltage at 2.5 kV positive ion
	EM voltage:	650 V
	Cone voltage:	25 V
	Peak:	501 ($M^{Cl37\ Cl37}$-H^+, 15%), 499 ($M^{Cl35\ Cl37}$-H^+ 86%), 497 ($M^{Cl35\ Cl35}$-H^+, 100%) *m/z*
TLC:	Conditions:	Kieselgel 60F_{254}. Dichloromethane/methanol/acetic acid (70/29/1) Single spot observed, R_f = 0.5-0.6. Visualisation with UV at 254 nm.
IR:	Instrument:	Bruker Alpha Platinum ATR
	Range:	4000-400 cm^{-1}, neat
	Peaks:	3302, 2890, 1681, 1603, 1516, 1346, 1052, 1025, 810 cm^{-1}
^{1}H NMR:	Instrument:	Bruker Avance III-500
	Field strength:	500 MHz
	Solvent:	D_2O (4.79 ppm)
	Spectral data:	δ 3.38 (1H, t, *J* = 8.2 Hz), 3.55 (2H, q, *J* = 9.0 Hz), 3.77 (1H, dd, *J* = 6.8, 4.0 Hz), 3.87 (1H, d, *J* = 9.1 Hz), 4.15 (1H, dd, *J* = 5.7, 10.9 Hz), 4.41 (1H, m), 4.50 (1H, d, *J* = 7.7 Hz), 5.25 (1H, d, *J* = 3.6 Hz), 6.24 (1H, s), 4.50 (1H, d, *J* = 7.7 Hz), 7.65 (2H, d, *J* = 8.8 Hz), 8.24 (2H, d, *J* = 8.7 Hz) ppm Isopropanol, diethyl ether, and acetic acid were quantified at 1.4%, 1.4%, and 0.2% mass fraction respectively by the ^{1}H NMR.
^{13}C NMR:	Instrument:	Bruker Avance III-500
	Field strength:	126 MHz
	Solvent:	D_2O (reference on isopropanol 24.4 ppm)
	Spectral data:	δ 56.0, 66.7, 69.5, 71.5, 72.2, 73.4, 75.9, 76.0, 103.2, 124.3, 128.0, 147.8, 149.0, 167.4, 174.8 ppm
Melting point:		148 °C decomposition
Microanalysis:	Found:	C = 37.4%; H = 4.1%; N = 4.7%; (January 2020)
	Calculated:	C = 40.9%; H = 4.0%; N = 5.6%; (Calculated for $C_{17}H_{20}N_2O_{11}Cl_2$)