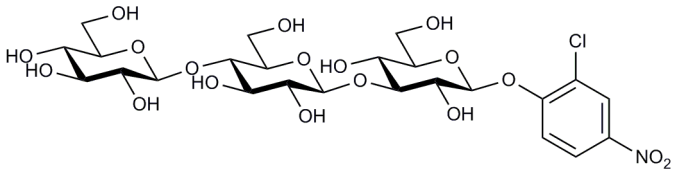
2-CHLORO-4-NITROPHENYL-β-(1,3:1,4)-GLUCOTRIOSIDE (Lot 1505DM3-174a)

O-CNPBG3 **05/15**

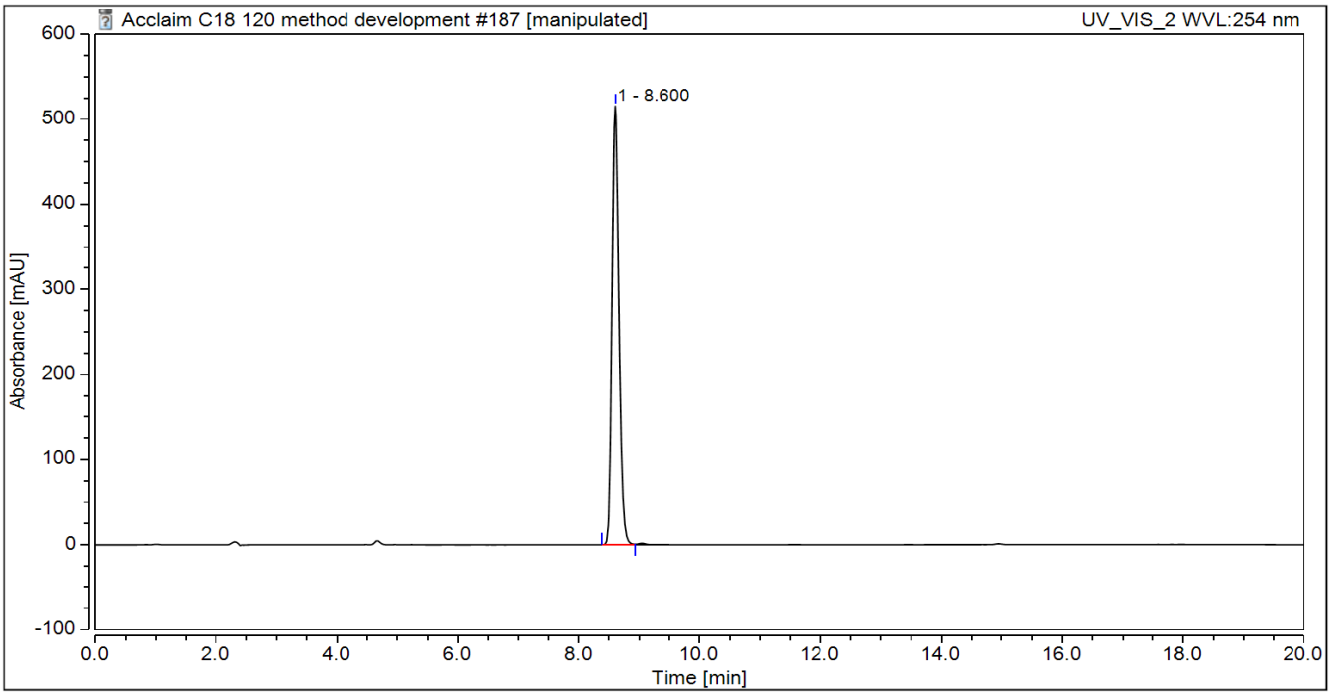
Synonym: 2-Chloro-4-nitrophenyl β-D-glucopyranosyl-(1 → 4)-β-D-glucopyranosyl-(1 → 3) -β-D-glucopyranoside
CAS: N/A
Molecular Formula: $C_{24}H_{34}ClNO_{18}$
MW: 660.0



PURITY: > 98%

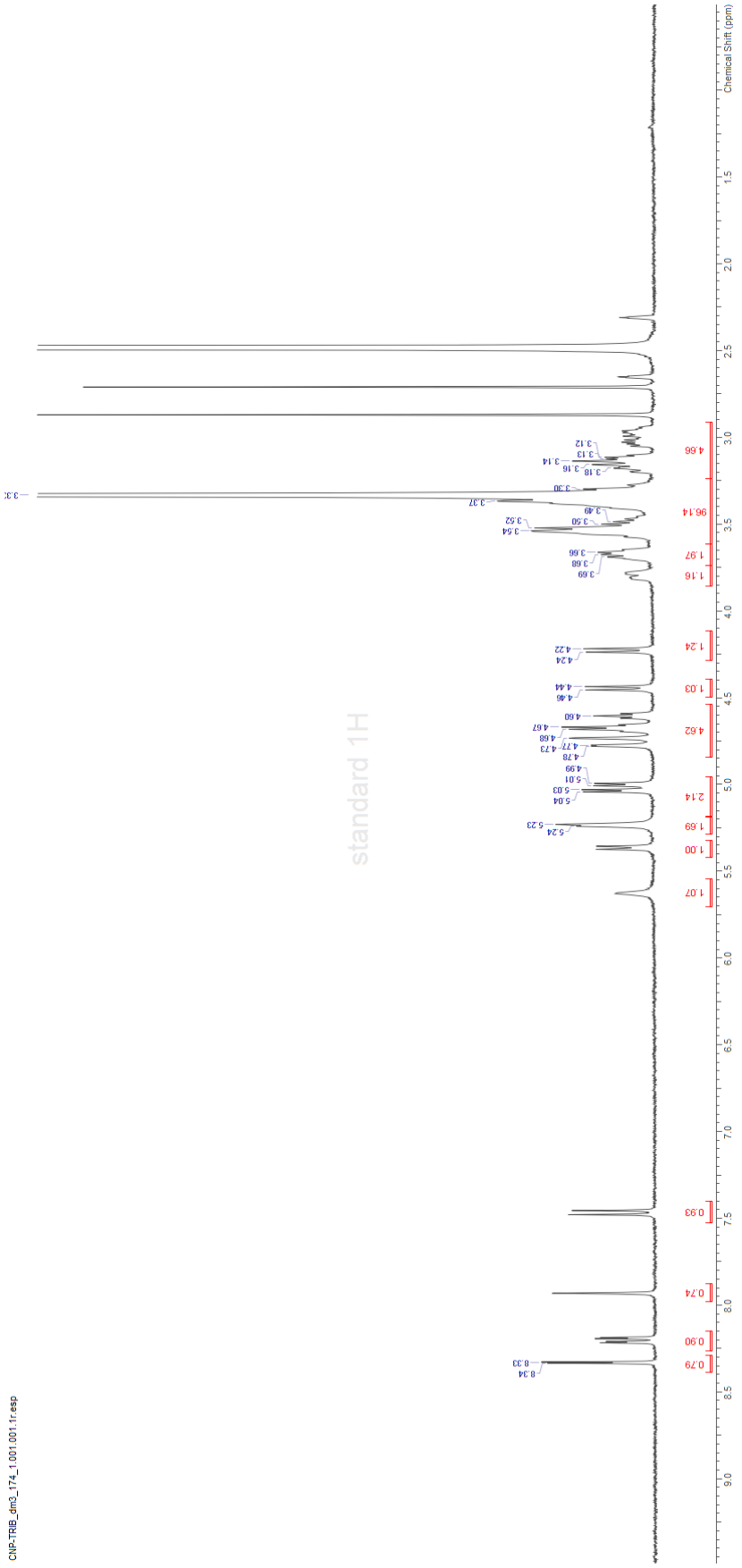
HPLC:
Column :- Acclaim 120 C18, 3 µm (3 x 150 mm)
Temperature :- 55°C
Flow rate :- 0.4 mL/min (Eluent gradient shown below)
Detector :- UV (256 nm)
HPLC System :- Thermofisher U3000 Ultimate and Chromeleon v 7.0 software

Time (min)	H_2O (%)	CH_3CN (%)
0	85	15
1	85	15
13	75	25
15	85	15
20	85	15



^{1}H-NMR:

A Brüker Avance 400 was employed for ^{1}H NMR spectra (400.13 MHz). Resonances, ∂, are in ppm units downfield from an internal reference in C_2D_6SO (∂_H = 2.50).



TYPICAL APPLICATION AS LICHENASE SUBSTRATE

SUBSTRATE SOLUTION PREPARATION:

Prepare substrate solution by dissolving 20 mg of 2-chloro-4-nitrophenyl-β-(1,3:1,4)-glucotrioside (cat. no. **O-CNPBG3**) in 150 µL of dimethylsulfoxide and diluting this solution to 3 mL with deionised H_2O (10 mM final concentration in 5% DMSO/H_2O). This is termed CNPBG3 Reagent Solution.

LICHENASE ASSAY PROCEDURE:

1. Dispense 0.1 mL aliquots of CNPBG3 Reagent Solution into test tubes and pre-incubate at 40°C for 3 min.
2. Pre-incubate lichenase solution at 40°C for 3 min.
3. To each tube containing CNPBG3 Reagent Solution (0.1 mL), add 0.1 mL of pre-equilibrated lichenase solution directly to the bottom of the tube. Incubate at 40°C for exactly 10 min (from time of addition).
4. At the end of the 10 min incubation period, add exactly 1.5 mL of 2% Tris solution (pH 10) and stir the tube contents vigorously.
5. Read the absorbance of the solutions and the reaction blank at 400 nm against distilled water.

CALCULATION OF ACTIVITY:

One Unit of activity is defined as the amount of enzyme required to release one micromole of 2-chloro-4-nitrophenol (CNP) from the CNPBG3 reagent in one minute under the defined assay conditions, and is termed a CNPBG3 Unit.

$$= \frac{\Delta E_{400}}{\text{Incubation Time}} \times \frac{\text{Total Volume in Cell}}{\text{Aliquot Assayed}} \times \frac{1}{E_{mM}} \times \text{Dilution}$$

ΔE_{400} = Absorbance (reaction) - Absorbance (blank)
Incubation Time = 10 min
Total Volume in Cell = 1.7 mL
Aliquot Assayed = 0.1 mL
E_{mM} of CNP (at 400 nm) in 2% Tris solution = 16.6
Dilution = Dilution of the original lichenase solution

EXAMPLE

Under the precise assay conditions described here, a 1:5000 dilution of non-specific *endo*-1,3(4) β-Glucanase (*Clostridium thermocellum*) (cat. no. **E-LICACT**, Lot# 90901b, 2500 U/mL) generated an absorbance value of 0.70. In this case, the calculation of lichenase activity in CNPBG3 Units becomes:

$$= \frac{0.70}{10} \times \frac{1.7}{0.1} \times \frac{1}{16.6} \times 5000$$

$$= 358 \text{ CNPBG3 U/mL}$$

In order to demonstrate the linearity of this assay format, the example incubation with non-specific *endo*-1,3(4) β-Glucanase (*Clostridium thermocellum*) (cat. no. **E-LICACT**) described above was also stopped at 3, 6, 9 and 12 min intervals to generate the graph shown (R^2 = 0.999).