

Partial Acetolysis

Natsuka, S., ed.
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Reference

Fluorescence Method for the structural analysis of oligomannose-type sugar chains by partial acetolysis.
Shunji Natsuka, Sumihiro Hase, Tokuji Ikenaka. *Anal. Biochem.*, **167**, 154-159 (1987).

0.5 – 10 nmol PA-oligosaccharide(s)

↓ peracetylation by 20 μ L of pyridine + 20 μ L of Ac_2O , 100°C, 15 min

↓ evaporate

↓ partial acetolysis by 20 μ L of Ac_2O , AcOH, H_2SO_4 (10:10:1), 37°C, 12 h

↓ add 4 μ L of pyridine

↓ evaporate

↓ 0.4 mL of 50% saturated NaHCO_3

↓ CHCl_3 extraction, x 3 times

CHCl_3 layer

↓ dry by anhydrous NaSO_4

↓ divide into two tubes

Tube 1. for reducing end fragment

↓ dry up

↓ dissolve in 100 μ l of hydrazine, anhydrous

↓ 100°C, 22 h

↓ evaporation with toluene

↓ 8 μ l of Ac_2O , 200 μ l of saturated NaHCO_3

↓ on ice, 30 min

↓ Dowex 50x2 (H^+), pH to 3

↓ wash with 10 x V of DDW

↓ elute with 6 x V of 1.5 M NH_4OH

↓ dry up

↓ HPLC analysis

Tube 2. for non-reducing end fragments

↓ dry up

↓ dissolve in 50 μ l of MeOH

↓ add 20 μ l of 0.2% NaOMe in MeOH

↓ r.t., 37°C, 30 min

↓ 1% AcOH pH to 3

↓ dry up

↓ 10 μ l of PAion reagent, 90°C, 60 min

↓ 35 μ l of reduction reagent, 80°C, 35 min

↓ 0.5 g of Dowex 50x2 (H^+), pH to 3

↓ pour into mini-column

↓ wash with 5 mL of DDW

↓ elute with 3 mL of 1.5 M NH_4OH

↓ co-evaporate with triethylamine, repeated

↓ paper electrophoresis

PA-saccharides

↓ HPLC analysis