Technical Bulletin

Procainamide Labeled Dextran Ladder

Catalogue number SMB01378

Product Description

Glycans are associated with numerous biological processes due to their structural and binding properties. New functional roles of glycans are constantly being discovered. However, many glycans are not assigned with any functional aspect. To better understand their roles, glycan profiling is essential.

Procainamide labelling of glycans is one method of profiling glycans by coupling with hydrophilic interaction liquid chromatography (HILIC) and fluorescence detection (FD) or mass spectrometry (MS).

The procainamide labeled dextran provides for assignment of GU retention values of unknown glycans to aid in identification and standardization over time.

Key benefits are listed below:

1. Each labeled glycan - Glucose Unit (GU) value increases in an incremental value of its GU thus helping an easy assignment of unknown glycans.
2. Assignment of sample glycans can be done against dextran ladder. GU retention values allows different instruments to be used, and sample comparisons can be made over time for quality control.
3. The observed GU values are between 2 to 30, thus enabling larger glycans retention profiling.
4. The ladder can also serve as a system suitability standard for each batch of samples.
5. Elution of glycans in ladder happens with increasing units, thus enabling the use on day-to-day analysis in LC.
6. A polynomial fit can then be used to assign RT values of sample glycans for identification or confirmation in a QC setting.

Components of procainamide labeled dextran ladder

*Procainamide labeled dextran ladder containing increasing GU units. Each vial contains 200 µg of lyophilized powder.

Precautions and Disclaimer

This product is for R&D use only. Not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Storage/Stability

Store the sealed vials at 4 °C.

Preparation Instructions

The lyophilized material consists of a broad range of both straight chains, α-1,6 glucose linkages, and larger branched chain oligosaccharides with α-1,3 linkages. The use of 75% acetonitrile (water) solubilizes the shorter chain GU components while use of 50% acetonitrile in water will solubilize many of the longer, branched chain components as well. Depending on the GU range of interest one may choose more, or less, acetonitrile for the reconstitution. See Figure 1 below.

One vial of procainamide labeled dextran ladder contains 200 µg of lyophilized powder. For reconstitution, dissolve the sample in 200 uL of 75% acetonitrile. Mix well and centrifuge to remove any undissolved material.
Note: Dilution can be adjusted depending on the needs of experiments.

Procedure

LC Conditions:

Column: BIOshell™ Glycan HPLC Column (150 mm x 2.1 mm x 2.7 µm) 90 Å.
Column oven temperature: 40 °C
Sample temperature: 5 °C
Flow rate: 0.3 mL/min
Eluent A: 75 mM Ammonium formate in water
Eluent B: ACN
Injection volume: 10 µL
Fluorescence detector: Ex: 308 nm Em: 359 nm
Gradient:

Time (min)	A [%]	B [%]
0	20	75
75	41	59

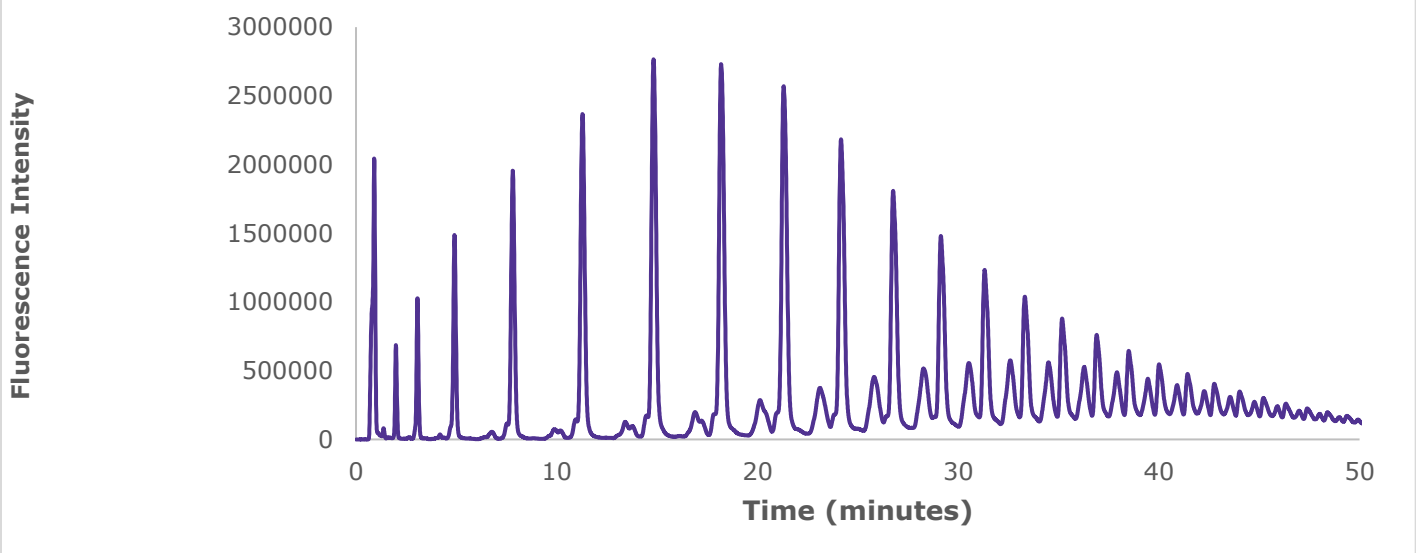
Figure 1: Chromatogram showing procainamide labeled dextran ladder, as measured by fluorescence. The solvent used to reconstitute the sample has an influence on the series of dextran components seen.

For details on labelling of released glycans using procainamide in the laboratory, please see the application note:

Step-by-Step Protocol for Procainamide Labelled Glycan Profiling of a Monoclonal Antibody

References

1. Klapoetke, S., *et al*., The evaluation of a novel approach for the profiling and identification of N-linked glycan with a procainamide tag by HPLC with fluorescent and mass spectrometric detection. *J Pharm Biomed Anal*,**53(3)**, 315-24 (2010).
2. HPLC Analysis of a Procainamide-Labeled Dextran Ladder on BIOshell™ Glycan using HILIC-FLR. (https://www.sigmaaldrich.com/IN/en/technical-documents/chromatograms/hplc/hplc-analysis-of-a-procainamide-labeled-dextran-ladder-on-bioshell-glycan-using-hilic-flr/supelco/g006408)



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