

Technical Bulletin

2-AB Labeled Dextran Ladder

Catalogue number SMB01377

Product Description

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

Use of fluorescently labeled glycan is one of the preferred methods for profiling glycans in combination with Hydrophilic Interaction Liquid Chromatography (HILIC) with fluorescence detection.

The 2-AB (2-Aminobenzamide) labeled dextran ladder provides a relative retention index, in glucose units or GU to aid in proper assignment of glycan structure.

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 2-AB 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.

Components of 2-AB labeled dextran ladder

*2-AB labeled dextran ladder containing increasing glucose chain length provided as 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

One vial of 2-AB labeled dextran ladder contains 200 µg of lyophilized powder. For reconstitution, the sample can be dissolved in 100 µL of water and 100 µL of acetonitrile for a final concentration of 1µg/mL.

Note: Dilution can be adjusted depending on the needs of experiments. It may be useful to centrifuge the sample if any insoluble material is observed.

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

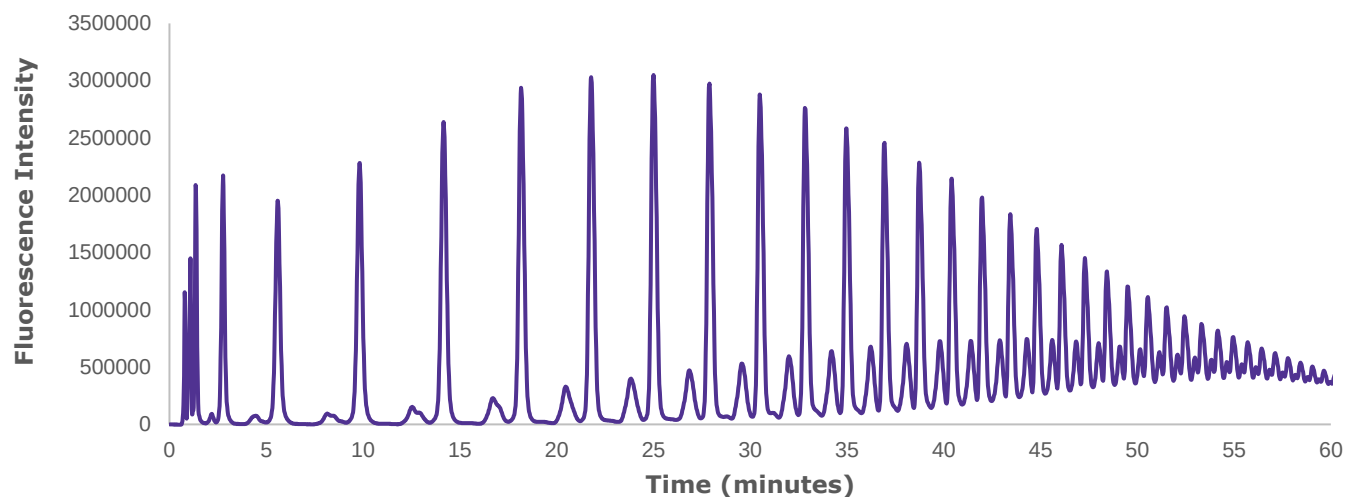
Injection volume: 5 µL

Fluorescence detector: Ex: 330 nm Em: 420 nm

Gradient:

Time (min)	A [%]	B [%]
0	25	75
40	40	60
45	40	60

Figure 1: A chromatogram showing the fluorescence of dextran ladder with increasing gradient over time.



References

1. Bigge, J.C., *et al.*, Nonselective and Efficient Fluorescent Labelling of Glycans Using 2-Amino Benzamide and Anthranilic Acid. *Anal. Biochem.*, **230 (2)**, 229-238 (1995).
2. Wuhler M, *et al.*, Two-Dimensional HPLC separation with Reverse-Phase-Nano-LC-MS/MS for the Characterization of Glycan Pools After Labelling with 2- Aminobenzamide. *Methods Mol Biol.*, **534**,79-91(2009).
3. Ahn J, *et al.* Separation of 2-Aminobenzamide labeled glycans using hydrophilic interaction chromatography columns packed with 1.7 μm sorbent. *Journal of Chromatography. B*, **878(3-4)**, 403-408 (2010).

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smb01377pis Rev 02/24

