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ProductInformation

Dextran Ladder
Glycan standard for HPLC

Product Code **D3818**
Storage Temperature –20 °C

TECHNICAL BULLETIN

Product Description

One of the distinguishing features of the proteome in eukaryotic cells is that most proteins are subject to post-translational modifications, of which glycosylation is the most common form. It is estimated that more than half of all proteins are glycoproteins. Various methods are available to remove the carbohydrate (glycan) groups from glycoproteins for further study of the separated proteins and glycans. Once the glycans have been released from the glycoprotein, the glycan pool can be analyzed by various techniques. One method involves fluorescent labeling of the glycans and analysis by normal phase HPLC. In order to calibrate the HPLC system, partially hydrolyzed dextran, consisting of a variable number of monomeric glucose units, is used as an external standard. This dextran standard gives a characteristic ladder profile from monomeric glucose to approximately a 20-mer of glucose oligosaccharide, depending on the chromatographic conditions employed. The elution position of each peak in this ladder is expressed as a glucose unit (gu) and is used to assign gu values to peaks in the released glycan pool.

The Dextran Ladder is prepared by partial acid hydrolysis of dextran from *Leuconostoc mesenteroides* with an average molecular weight of 100-200 kDa. A mixture of α-1→6 linked glucose oligosaccharides of various lengths is produced. The purity and structural integrity of the ladder is assessed by fluorescently labeling an aliquot and subsequent analysis by normal phase HPLC. The separation of the different glucose oligomers on an amide HPLC column is shown in Figure 1.

Precautions and Disclaimer

This product is for laboratory use only. Not for drug, household, or other uses. Consult the MSDS for information regarding hazards and safe handling practices. It is recommended to read the entire technical bulletin prior to starting the procedure.

Preparation Instructions

One vial of the Dextran Ladder contains 200 µg of dried, partially hydrolyzed dextran. Allow the unopened vial to reach room temperature and ensure that most of the material is at the bottom of the vial by gently tapping on a solid surface. Gently remove the cap, reconstitute with the desired volume of water or buffer, replace the cap, and mix thoroughly. Centrifuge briefly before use.

The Dextran Ladder may be fluorescently labeled with Sigma's GlycoProfile™ 2-AB or 2-AA Labeling Kits. The GlycoProfile labeling kits are designed for efficient labeling of glycans with either 2-aminobenzamide (2-AB) or 2-aminobenzoic acid (anthranilic acid; 2-AA) by reductive amination. The fluorophores increase the spectral activity of the glycans, improving detection by HPAE (high-performance anion exchange chromatography), HPLC, and ESI-MS (electrospray mass spectroscopy) methods. GlycoProfile Glycan Clean-Up Cartridges may be used for clean up of glycan samples after reductive amination labeling or enzymatic digestion.

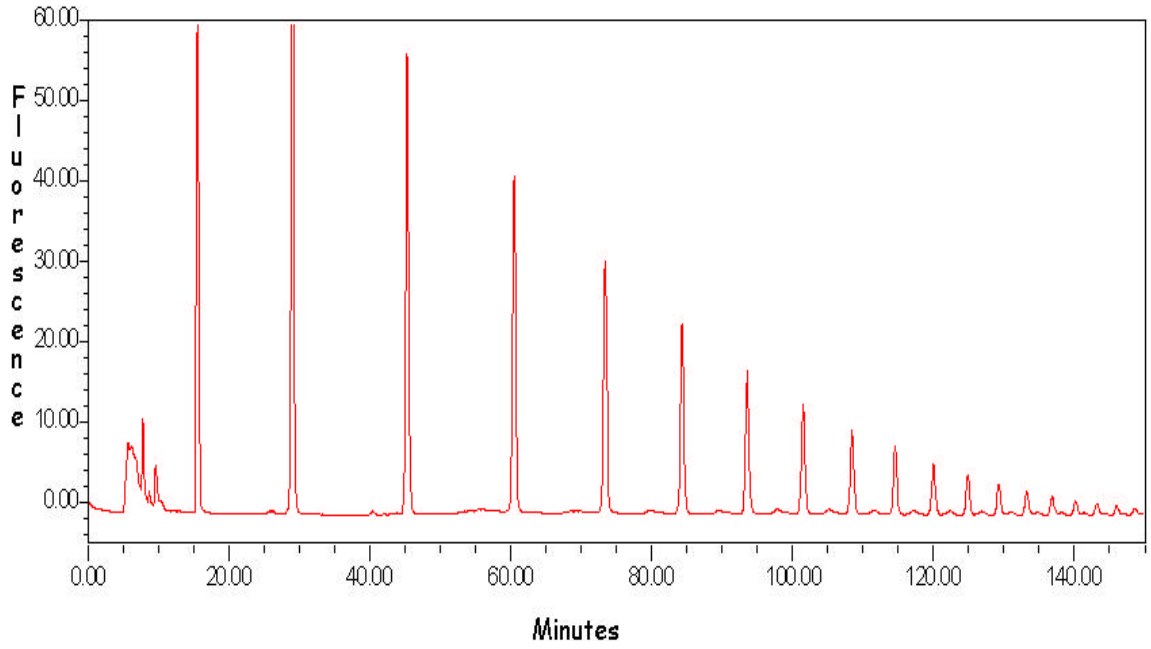
During handling, avoid the use of powdered safety gloves or of any glass or plasticware that may be contaminated with glycosidases or environmental carbohydrates.

The 200 µg of the Dextran Ladder is sufficient for 10 to 50 normal phase HPLC chromatographs, as fluorescence detection is more sensitive than absorbance. The actual number of tests per vial is dependent on the yield of the labeling reaction and the amount loaded onto the HPLC column.

Storage/Stability

The product ships on wet ice and storage at –20 °C is recommended. After reconstitution, store both unlabeled and labeled aliquots at –20 °C.

Figure 1.
Normal phase HPLC chromatograph of Dextran Ladder after fluorescent labeling with 2-AB.[1]



Related Products

GlycoProfile 2-AB Labeling Kit,
Product Code PP0520
GlycoProfile 2-AA Labeling Kit
Product Code PP0530
GlycoProfile Glycan Clean-Up Cartridges
Product Code G8169

Reference

1. Guile, G.R., *et al*., A rapid high-resolution high-performance liquid chromatographic method for separating glycan mixtures and analyzing oligosaccharide profiles. Anal. Biochem., **240**, 210-226 (1996).
2. Yamashita, K., *et al*., Analysis of Oligosaccharides by Gel Filtration, Meth. Enzymol, **83**, 105-126 (1983).

Labeling of glycans with 2-AB is covered under U.S. Patent No 5,747,347 and its foreign equivalents.

AE,NA,VNC,MAM 06/06-1