Certificate of Analysis

Fetuin Glycoprotein Standard

Cat. #: GCP-FET-50U-X4 (GCP-FET-50U B95H-05 *4) Batch: B98G-02

Nominal size: 4 * 50µg Expiry Date:17 May 2024

Description: A glycoprotein standard for use during glycan release and labeling.

Source: This product is purified from fetal calf serum. Fetuin is a glycoprotein present in the circulation which is synthesized by hepatocytes. Fetuin exists in a variety of glycoforms containing bi-, tri-, and tetra-antennary oligosaccharides with variable sialylation.

Form: Dry. Lyophilised powder.

Molecular Weight: 36 kDa (protein weight only)

Amount: 29 µg protein (In comparison to BSA standard, determined by BCA assay. Value rounded to nearest µg)

Storage: Refrigerate (-20°C) both before and after dissolving. This product is stable for at least 5 years as supplied.

Shipping: The product is shipped at ambient temperature.

Handling: Once dissolved avoid repeated thawing and refreezing, storage over 3 h at room temperature or above, exposure to light and long term exposure to acid as these will cause glycan desialylation.

Safety: This product is non-hazardous and has been purified from natural sources certified to be free of all hazardous material including pathogenic biological agents.

For research use only. Not for human or drug use

Analysis

Fetuin glycans were released from Fetuin Glycoprotein (Cat# GCP-FET-50U) using PNGaseF.

Following release the glycans were labeled using 2-Aminobenzamide (2-AB) using the LudgerTag™ 2-AB Glycan Labeling Kit (Cat# LT-KAB-A2).

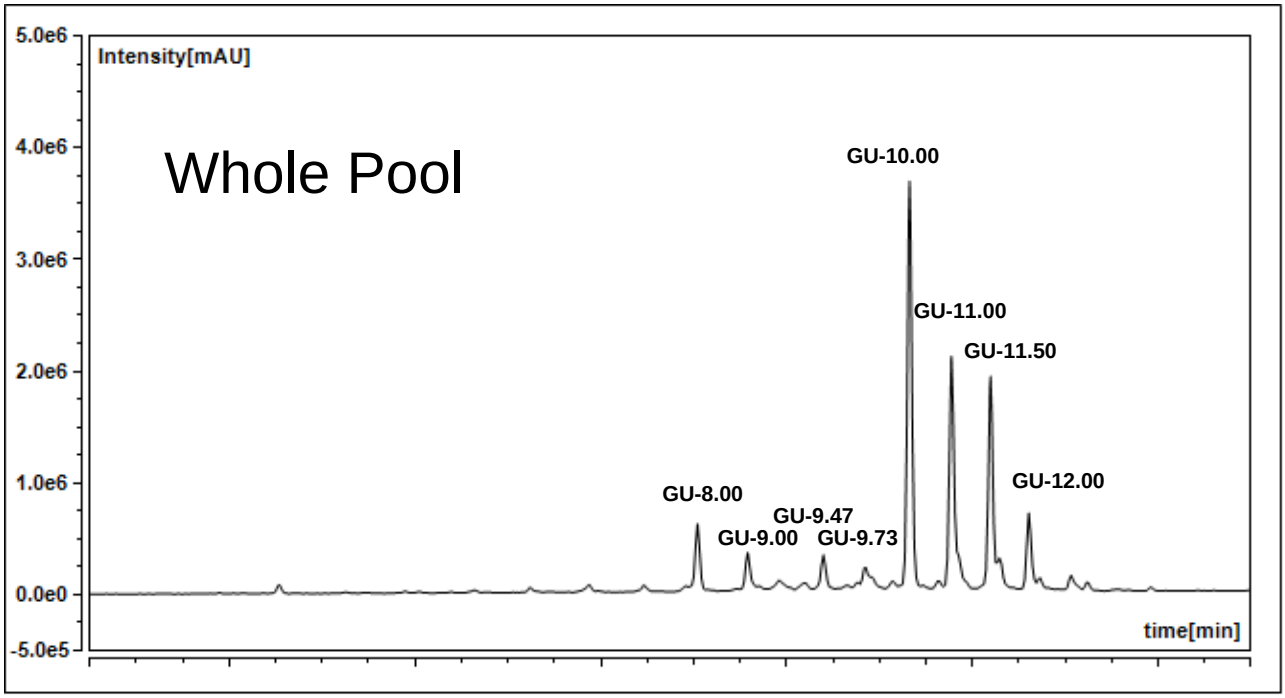

Figure 1. *HILIC HPLC profile of 2-AB labeled Fetuin N-glycans, released by PNGase F from GCP-FET-50U batch B95H-05 run on Waters BEH Glycan column.*

Figure 1 shows a LudgerSepN2 HPLC profile of bovine fetuin N-glycans. To thoroughly investigate the N-glycans we first separate them based on charge on a LudgerSepC3 column (Figure 2) and then run each fraction on a LudgerSepN2 column. From these studies, combined with exoglycosidase investigation we identified the glycans shown in Table 1. For further information on Glycoprofiling please contact us at info@ludger.com

Sialic acid analysis of GCP-FET-50U

Quantitative sialic acid analysis was performed on 3 separate vials of GCP-FET-50U using the LudgerTag™ DMB sialic acid labelling kit (LT-KDMB-A1). The labelled sialic acid chromatography was performed on a HPLC equipped with a LudgerSep R1 column (LS-R1-4.6x150).

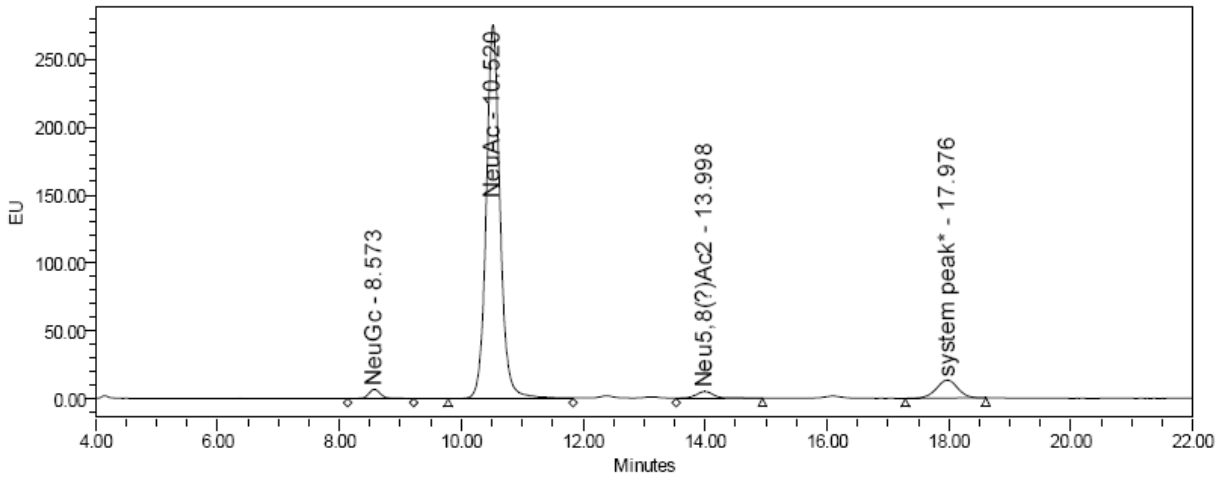

Figure 2. LudgerSep-uR2 HPLC profile of 1,2-diamino-4,5-methylenedioxybenzene.2HCl (DMB) labelled Neu5Ac of acetic acid hydrolysed GCP-FET-50U (Batch B95H-05).

The expected value of NeuAc in fetuin glycoprotein is 314nmol/mg protein (see page 1 for protein amount result).

Quantity of NeuAc per mg fetuin protein from test samples = 319.03 ± 10.8 nmols/mg (9.25 nmol)

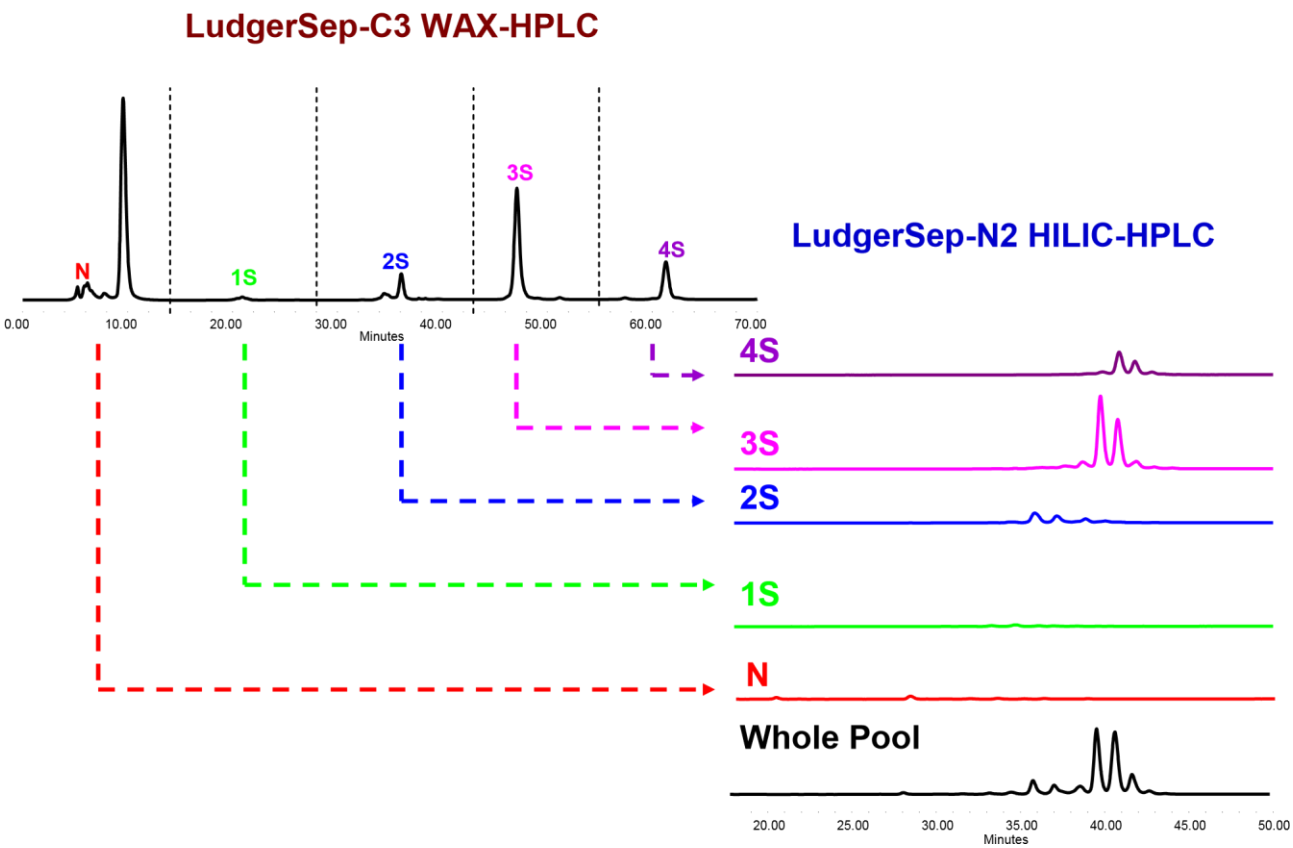

Figure 3. *LudgerSep-C3 profile and subsequent LudgerSepN2 analysis of bovine fetuin PNGaseF released N-glycans from a similar batch of GCP-FET.*

2-AB labelled glycans were separated on the LudgerSepC3 column and these fractions were then separated on the LudgerSepN2 column. This figure demonstrates the complexity of N-glycans present in the sample. A combination of LudgerSepC3/N2 and exoglycosidase digestion is required to identify the glycans and their relative abundance, as shown in Table 1. N- neutral glycans, 1S – monosialylated glycans, 2S – disialylated glycans, 3S – trisialylated glycans & 4S – tetrasialylated glycans.

Structure	GU	Whole Pool % Area
Bgd?	4.4	0.5
Bgd?	6.2	0.9
A2G(3)2	7	0.4
A2G(4)2	7.1	
A3G(4)2?	7.5	0.7
A2G(4)2S(6)1	7.9	1.4
A3G(4,4,3)3	8.3	6.8
A3G(4)3	8.3	
S2	8.5	5.3
A3G(4,4,3)3S1	8.6	
A3G(4)3S1	8.6	
S2	8.7	
S2	9.2	5.2
A3G(3,4)2S(3)3?	9.2	
S2	9.6	30.6
A3G(4)3S(?)3	9.6	
A3G(4,4,3)3S(3,?,?,?)4	9.6	
A3G(4)3S(?)3	10	33.4
S2	10	
A3G(4,4,3)3S(3,?,?,?)4	10	
A3G(4)3S(?)3	10.4	12.0
A3G(4,4,3)3S(3,?,?,?)4	10.4	
A3G(4)3S(?)3	10.8	2.3
A3G(4,4,3)3S(3,?,?,?)4	10.8	
A3G(4,4,3)3S(3,?,?,?)4	11.3	0.5
S2 structures include:		
A2G(3)2S(?)2		
A2G(4)2S(?)2		
A3G2S(?)2		
A3G(4,4,3)3S(?)2		

Sialylated state	Relative Percentage (%)
Neutral	4
Monosialylated	2
Disialylated	13
Trisialylated	61
Tetrasialylated	20

Table 1. *Summary of bovine fetuin N-glycans found in a similar batch of GCP-FET.*

See the end of this document for details of the glycan nomenclature used. A ? symbol indicates the linkage type is unknown. Bgd? – non-glycan peak, GU – glucose units – a system of comparing glycans to a glucose homopolymer standard. Many common N-glycans have reported GU values. A combination of GU value, mass spectrometry and exoglycosidase digestion (Figure 3), can be used to unambiguously identify most N-glycans.

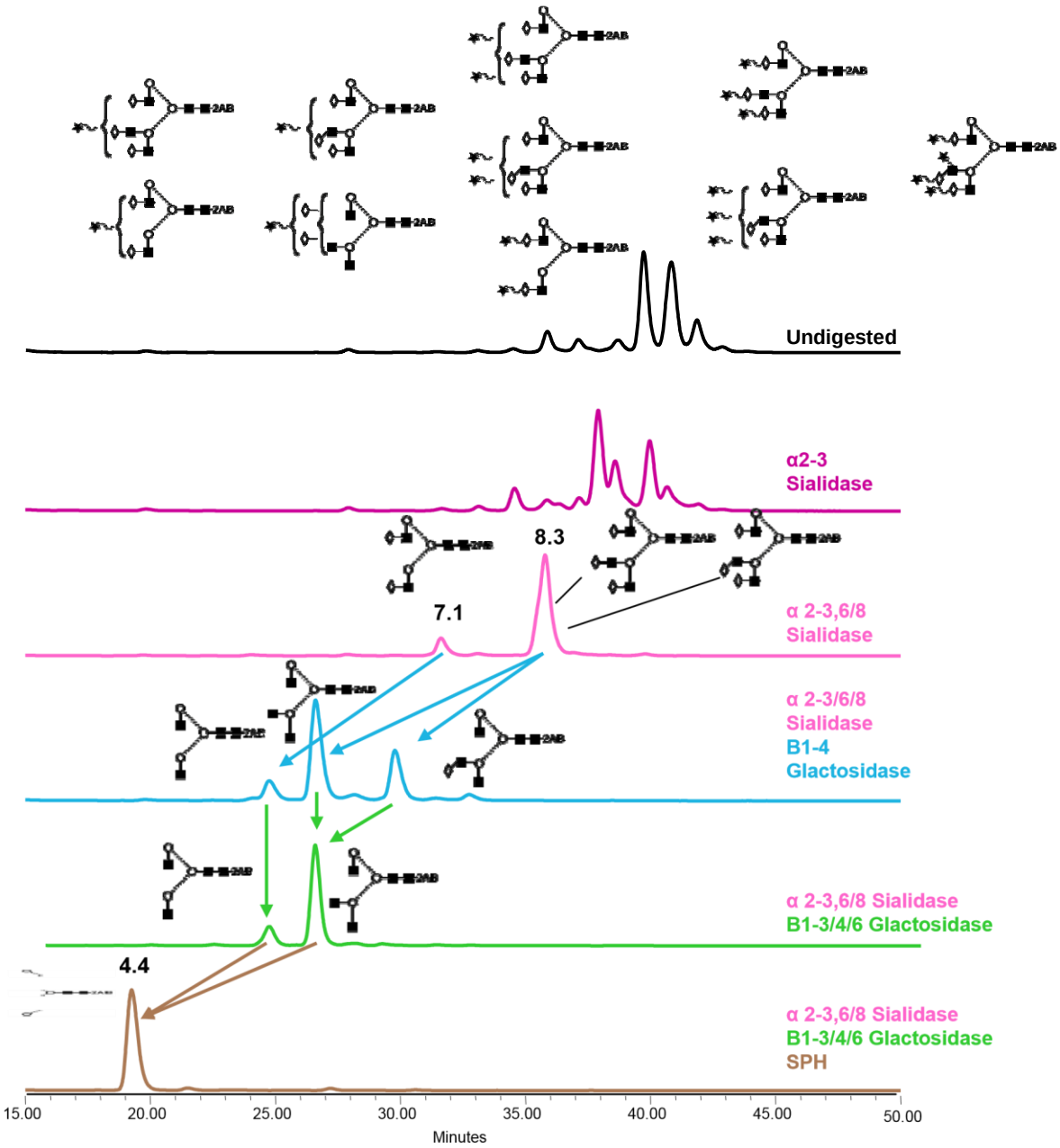

Figure 4. *Example exoglycosidase analysis of bovine fetuin N-glycans from a similar batch of GCP-FET.* *LudgerSepN2 chromatograms are shown.*

The major structures that are present
after removal of sialic acid are:

27% 53% 13%

Figure 5. *Relative amount of each core type of N-glycan, after removal of sialic acids, from a similar batch of GCP-FET.*

Structure Abbreviations

All N-glycans have two core GlcNAcs; F at the start of the abbreviation indicates a core fucose, (6) after the F indicates that the fucose is α1-6 linked to the inner GlcNAc; Mx, number (x) of mannose on core GlcNAcs; Ax, number of antenna (GlcNAc) on trimannosyl core; A2, biantennary with both GlcNAcs as β1-2 linked; A3, triantennary with a GlcNAc linked β1-2 to both mannose and the third GlcNAc linked β1-4 to the α1-3 linked mannose; A3', triantennary with a GlcNAc linked β1-2 to both mannose and the third GlcNAc linked β1-6 to the α1-6 linked mannose; A4, GlcNAcs linked as A3 with additional GlcNAc β1-6 linked to α1-6 mannose; B, bisecting GlcNAc linked β1-4 to β1-3 mannose; Gx, number (x) of linked galactose on antenna, (4) or (3) after the G indicates that the Gal is β1-4 or β1-3 linked; [3]G1 and [6]G1 indicates that the galactose is on the antenna of the α1-3 or α1-6 mannose; Sx, number (x) of sialic acids linked to galactose; the numbers 3 or 6 in parentheses after S indicate whether the sialic acid is in an α2-3 or α2-6 linkage.

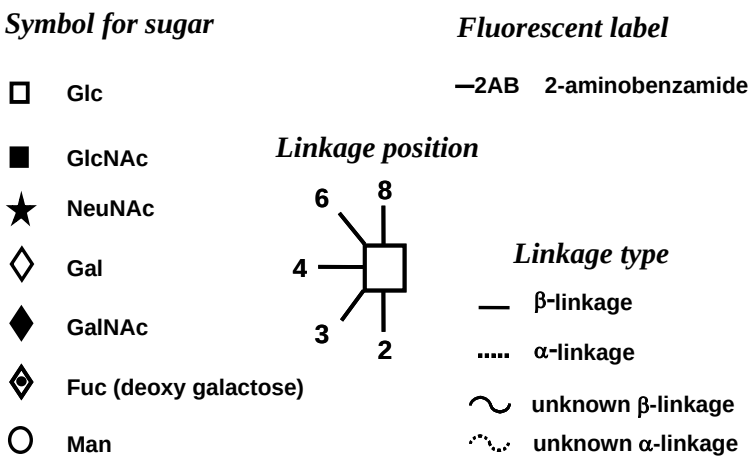

Figure 6. *Symbols used to depict glycan structures*

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This product is intended for *in vitro* research only.

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