

BIOshell™ Glycan HPLC Columns

Empowering the Analysis of Glycoproteins with Exceptional Reproducibility

BIOshell™ U/HPLC Columns

Characterizing and monitoring the glycosylation pattern of a biotherapeutic protein is required by regulatory authorities due to the fact that safety, efficacy and the serum half-life of therapeutic proteins can be affected by differences in their glycosylation pattern. Analysis and identification of glycoproteins can be challenging, however, because of the structural complexity of N-linked and O-linked sugar molecules. Hydrophilic interaction liquid chromatography (HILIC) is a proven technique for the separation and quantitation of isolated glycans under native conditions or after their derivatization with fluorescent labels.

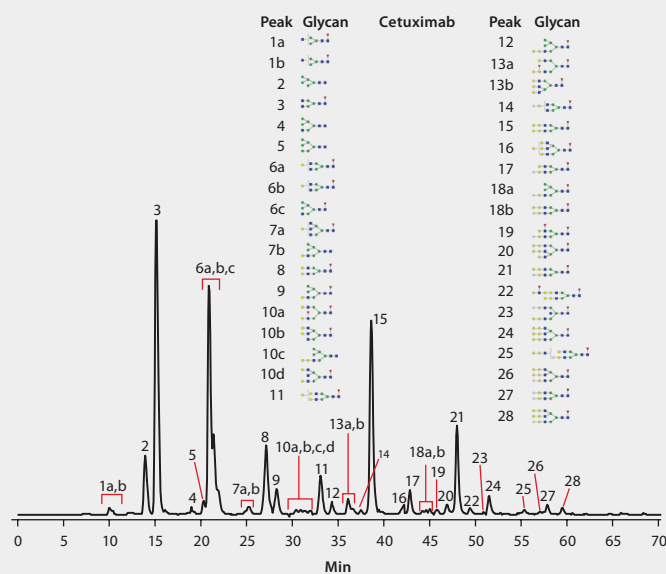
BIOshell Glycan HPLC columns are specifically engineered to deliver fast, high resolution, reproducible glycan separation using HILIC chromatography. There are many advantages to using Fused-Core® BIOshell Glycan HPLC columns for glycoprotein analysis:

- **Increased resolution, faster separations, and lower back pressure** – BIOshell HPLC columns utilize Fused-Core particle technology which offers significant performance benefits over traditional columns based on totally porous particles
- **Excellent reproducibility** – Quality control testing requires tight retention time and peak width specifications ensuring lot-to-lot reproducibility
- **Complimentary Sigma-Aldrich® products** – Sigma-Aldrich supplies reagents and consumables needed for each step in glycoprotein analysis as indicated in **Table 1**.

Steps in Glycoprotein Analysis



Figure 1. BIOshell Glycan Column Separation of Procainamide Labeled Cetuximab Glycans

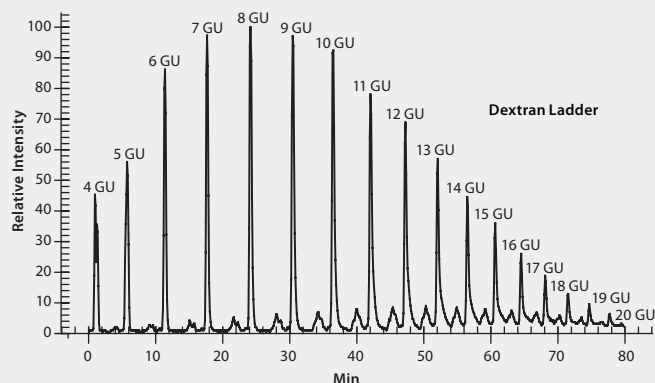


Cetuximab is a chimeric mouse-human IgG1 monoclonal antibody against the epidermal growth factor receptor. It is used to treat head, neck, and colorectal cancers. The antibody is N-glycosylated both in the Fc and Fab regions, which have been shown to impact safety and quality of the drug. Thus, characterizing its glycosylation pattern is exceptionally important. As shown in this application, the BIOshell Glycan column is able to elucidate the complex glycosylation of this biotherapeutic, allowing a better understanding of the drug's efficacy.

For a complete protocol detailing glycoprotein analysis, including testing conditions, visit sigma-aldrich.com/BIOshell

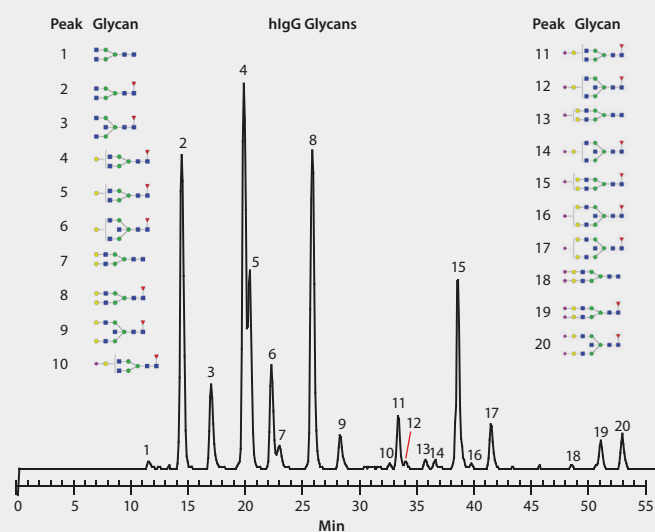
BIOshell™ Glycan Applications

Figure 2. BIOshell Glycan Column Separation of a Procainamide Labeled Dextran Ladder



The dextran ladder is used as an external standard for the analysis of glycans by HILIC mode HPLC after fluorescent labeling. When analyzed on the BIOshell Glycan HPLC column, this standard gives a characteristic ladder profile from monomeric glucose to a 20-mer glucose oligosaccharide. This ladder provides calibration reference points that can aid in identifying more complex glycans based upon retention characteristics.

Figure 3. BIOshell Glycan Column Separation of Procainamide Labeled Human IgG Glycans



A sample of human IgG glycans was analyzed on a BIOshell Glycan HPLC column resulting in the identification of 20 distinct peaks. Glycans were identified by mass spectrometry, which was coupled in line with the HPLC-fluorescence detector system. Excellent separation as well as symmetrical peak shape can be observed in the chromatogram.

Get Started

Additional resources are available for helping you implement BIOshell Glycan columns into your laboratory.

For product information, webinars, ordering and real time availability information, visit sigma-aldrich.com/BIOshell

Table 1. Sigma-Aldrich Reagents and Consumables for Glycoprotein Analysis

Description	Cat. No.
Step 1: Glycan Release	
IgG from human serum	14506
Trizma® HCl	T5941
Urea	U0631
Ammonium Bicarbonate	9830
PNGase F	7367
Step 2: Procainamide Labeling	
Sodium Cyanoborohydride	156159
Procainamide Hydrochloride	P9391
Dimethyl Sulfoxide	D8418
Acetic Acid	320099
Water	39253
Dextran Hydrolysate	31417
Step 3: Cleanup	
Acetonitrile	34851
DPA-6S 50 mg Cartridges	52624-U
Step 4: LC-MS Analysis	
BIOshell Glycan, 15 cm x 2.1 mm I.D., 2.7 µm	50994-U
Ammonium Formate	17843
Formic Acid	94318

BIOshell Glycan Fused-Core Silica Characteristics

- Pore Size – 90 Å
- Max Temp. – 65 °C
- Pressure – 1,000 bar (14,500 psi)
- Operating pH Range – 2-9
- Surface Area – 135 m²/g

BIOshell Glycan Fused-Core® HPLC Columns

Particle Size	I.D.	Length	Cat. No.
2.7 µm	2.1 mm	10 cm	50993-U
2.7 µm	4.6 mm	10 cm	50998-U
2.7 µm	2.1 mm	15 cm	50994-U
2.7 µm	4.6 mm	15 cm	50999-U
2.7 µm	2.1 mm	5 cm	50991-U
2.7 µm	4.6 mm	5 cm	50997-U

Our technical service staff is ready to answer questions.

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A technical seminar can be arranged on-site or via the web.
Request via seminars@sial.com