BIO-MAGHILIC



BIO-MAGHILIC is an innovative magnetic HILIC-based sorbent developed by BIO-DESIGN for the efficient purification and analysis of glycans. Designed with a focus on speed, reproducibility, and cost-effectiveness, BIO-MAGHILIC provides a powerful alternative to conventional solid-phase extraction methods in glycomics and biopharmaceutical applications.

This product enables highly selective enrichment and release of glycans from glycoproteins and monoclonal antibodies, offering a robust tool for researchers and quality control laboratories. By combining magnetic particle technology with hydrophilic interaction chromatography (HILIC) principles, BIO-MAGHILIC allows for simplified handling, minimal sample loss, and excellent reproducibility across multiple analyses.

Key Advantages:

- Magnetic separation ensures rapid and user-friendly workflows without the need for centrifugation or vacuum systems.
- High specificity for glycans, suitable for use with monoclonal antibodies and other glycoproteins.
- Compatible with fluorescence and mass spectrometry detection (FLD/MS), ensuring high sensitivity and strong signal intensity.
- Cost-effective solution, reducing reliance on expensive consumables and complex instrumentation.
- Scalable for both research and industrial applications, from academic laboratories to biopharmaceutical quality control.

App. Note-1: Human Serum N-Glycan Analysis Using Bio-MagHILIC

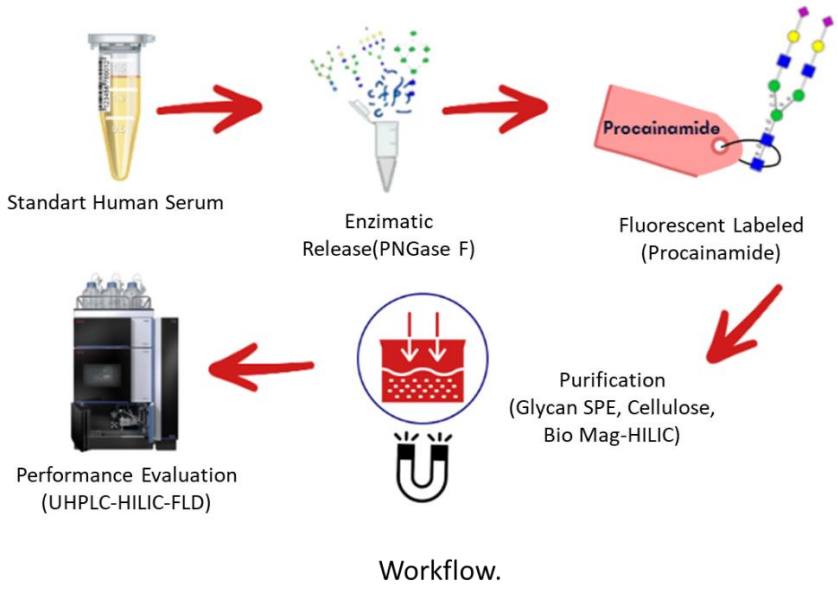
Introduction

Human serum contains a diverse pool of glycoproteins, many of which carry complex N-glycans that are functionally relevant in health and disease. Reliable and reproducible analysis of serum N-glycans is essential for biomarker discovery, disease classification, and translational medicine. Conventional purification methods, such as cellulose or solid-phase extraction (SPE), are often associated with limited recovery and high variability. Magnetic HILIC-based sorbents, such as **Bio-MagHILIC (BIO-DESIGN, Türkiye)**, provide an innovative alternative that combines efficiency, reproducibility, and user-friendly handling.

Objective

To evaluate the performance of Bio-MagHILIC for the purification and analysis of human serum N-glycans in comparison to Glycan-SPE and cellulose-based approaches.

Methods



Workflow.

Figure 1. Workflow for human serum N-glycan analysis. N-glycans were enzymatically released from standard human serum using **PNGase F**, followed by **procainamide fluorescent labeling**. Labeled glycans were then purified using three approaches: **Glycan-SPE (Sigma, Germany), Cellulose (Sigma, Germany), and Bio-MagHILIC (BIO-DESIGN, Türkiye)**. The performance of each method was subsequently evaluated by **UHPLC-HILIC-FLD**.

1. **Sample Source**: Standard human serum was used as the model glycoprotein mixture.
2. **Enzymatic Release**: N-glycans were enzymatically cleaved from serum glycoproteins using PNGase F under denaturing conditions.
3. **Fluorescent Labeling**: Released glycans were fluorescently labeled with procainamide to enhance sensitivity for fluorescence detection and improve MS ionization efficiency.
4. **Purification**: Labeled glycans were purified using three approaches:
    - Glycan-SPE (Sigma, Germany)
    - Cellulose-based method (Sigma, Germany)
    - Bio-MagHILIC (BIO-DESIGN, Türkiye)
      Magnetic separation was applied for Bio-MagHILIC.
5. **Analysis**: Purified glycans were analyzed by UHPLC-HILIC-FLD. Signal intensity and coefficient of variation (%CV) were used to compare recovery and reproducibility.

Results

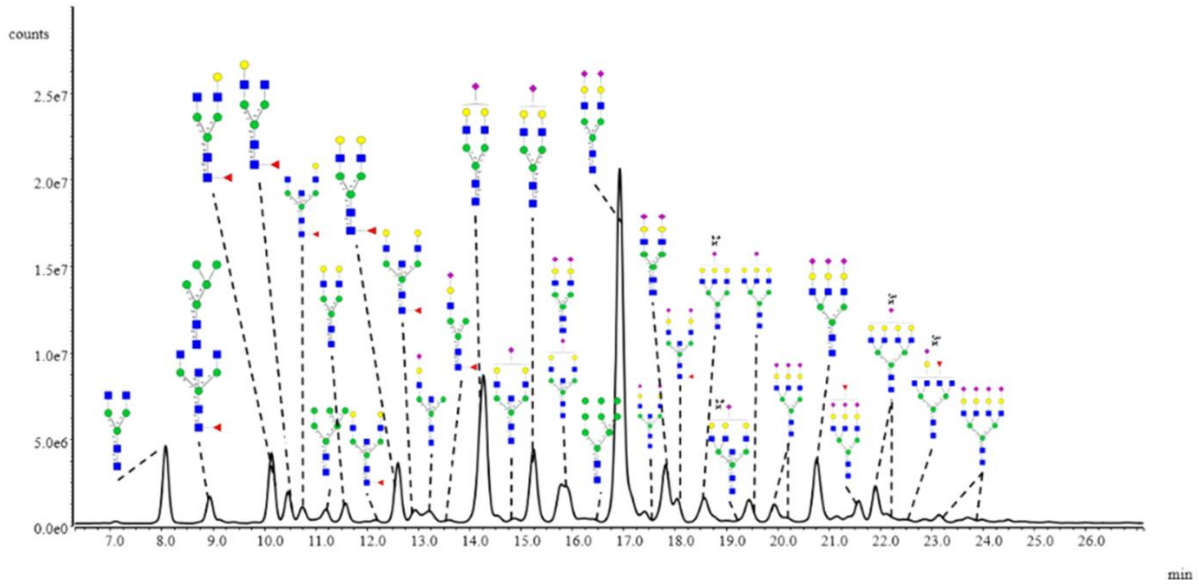


Figure 2. **Representative UHPLC-HILIC-FLD chromatogram of** N-glycans released from human serum glycoproteins **following PNGase F digestion, procainamide labeling, and purification with Bio-MagHILIC. Peaks correspond to distinct N-glycan species, with structural assignments indicated by glycan cartoons. The profile demonstrates the structural diversity of serum N-glycans, including core-fucosylated, galactosylated, sialylated, and bisected variants.**

- **Recovery**: Bio-MagHILIC provided the highest total glycan signal compared to cellulose and Glycan-SPE methods.
- **Reproducibility**: %CV values were lowest for Bio-MagHILIC, indicating high consistency across replicates.
- **Coverage**: A broad range of human serum N-glycan structures (fucosylated, galactosylated, and sialylated) were detected with strong signal intensities.

Conclusion

Bio-MagHILIC enables **efficient, reproducible, and high-sensitivity purification of human serum N-glycans**, outperforming conventional cellulose and Glycan-SPE methods. This workflow offers a powerful platform for glycomics research, biomarker discovery, and clinical translational studies.

App. Note-2: Efficient IgG *N*-Glycan Analysis Using Bio-MagHILIC

Introduction

Immunoglobulin G (IgG) is one of the most clinically relevant glycoproteins, and its N-glycosylation pattern strongly influences therapeutic efficacy, stability, and immune response. Comprehensive and reproducible N-glycan profiling is therefore essential in both academic glycomics research and the biopharmaceutical industry. Traditional glycan purification approaches, such as cellulose or SPE cartridges, often suffer from limitations in recovery efficiency and reproducibility.

Objective

To demonstrate the performance of **Bio-MagHILIC**, a magnetic HILIC-based sorbent developed by **BIO-DESIGN**, for the purification and analysis of IgG *N*-glycans.

Methods

- **Sample preparation**: Human IgG was subjected to enzymatic N-glycan release using PNGase F under optimized denaturing conditions with SDS and DTT.
- **Purification**: Released glycans were purified using Bio-MagHILIC particles via magnetic separation. Hydrophilic interaction chromatography principles allowed for selective glycan capture and efficient washing/elution.
- **Analysis**: Purified glycans were analyzed by HPLC-HILIC-FLD and LC-MS/MS. Structural assignment was performed using standard glycan libraries.

Results

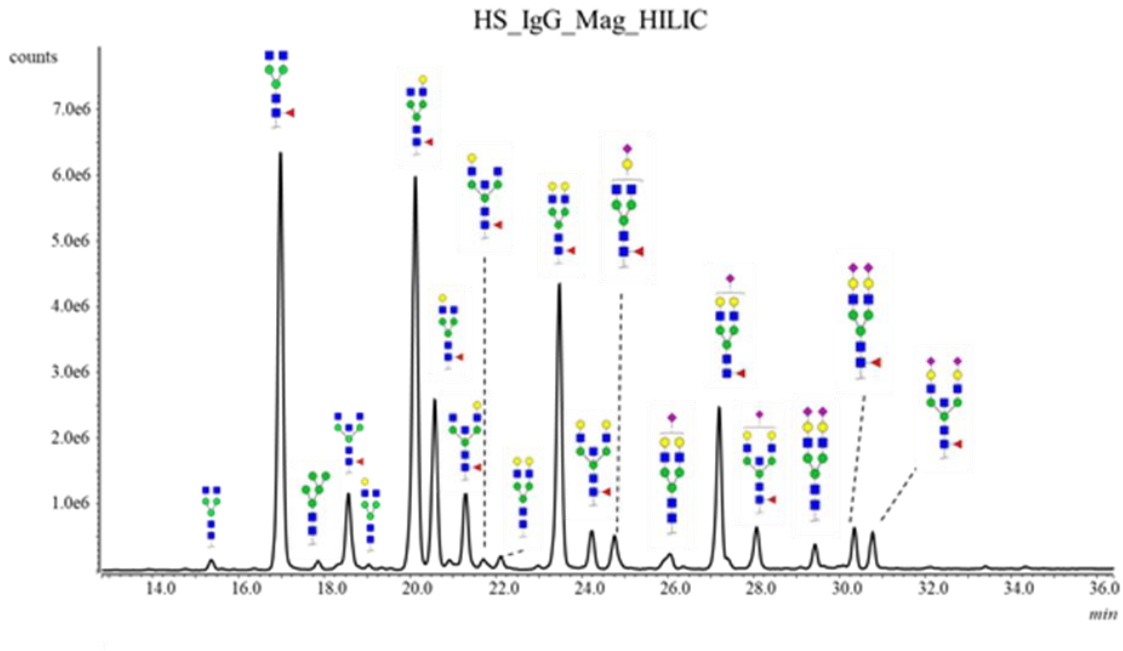


Figure 1. Representative N-glycan structures identified from human serum after purification with **Mag-HILIC (BIO-DESIGN, Türkiye)**. The profile includes a wide range of biantennary, galactosylated, sialylated, core-fucosylated, and bisected glycans. Structural diversity reflects the complexity of serum glycoproteins, and the results demonstrate the ability of Bio-MagHILIC to enrich and recover both major and minor glycan species with high sensitivity.

- **High Recovery**: Bio-MagHILIC showed superior glycan signal intensity compared to conventional cellulose and Glycan-SPE methods.
- **Reproducibility**: Coefficient of variation (%CV) was significantly lower with Bio-MagHILIC, ensuring consistent profiling across replicates.
- **Structural Coverage**: A wide range of IgG N-glycan species were detected, including core-fucosylated, galactosylated, sialylated, and bisected structures.

Conclusion

Bio-MagHILIC provides an efficient, reproducible, and user-friendly platform for IgG N-glycan analysis. Its high sensitivity and compatibility with both fluorescence and MS-based detection make it a valuable tool for glycomics research and monoclonal antibody characterization in biopharmaceutical development.

App. Note-3: Comparision of HILIC sorbents for *N*-glycan profiling by HPLC-HILIC-FLD

Aim

The aim of this study was to evaluate the performance of BIO-DESIGN's Bio-MagHILIC kit for glycan purification compared to conventional cellulose-based methods and commercial Glycan-SPE cartridges.

Introduction

Glycosylation plays a critical role in the structure and function of therapeutic glycoproteins, particularly monoclonal antibodies. Accurate glycan analysis requires efficient and reproducible purification methods. While cellulose and solid-phase extraction (SPE) approaches are commonly used, they often suffer from limitations in yield and reproducibility. To address these challenges, magnetic HILIC-based sorbents, such as Bio-MagHILIC, have been developed to provide a faster, cost-effective, and user-friendly alternative.

Methods

Model glycoproteins containing *N*-glycans were subjected to enzymatic release using PNGase F under optimized conditions with SDS and DTT. Released glycans were purified using three different approaches: cellulose (Sigma, Germany), Glycan-SPE (Sigma, Germany), and Bio-MagHILIC (BIO-DESIGN, Türkiye). Purified glycans were subsequently analyzed by HPLC-HILIC-FLD and evaluated in terms of total signal intensity and coefficient of variation (%CV) to assess recovery efficiency and reproducibility.

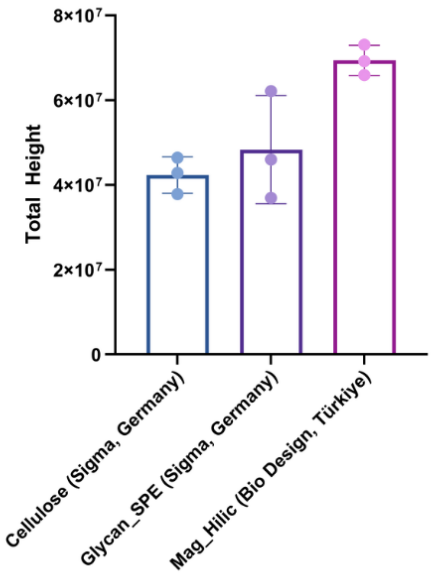


Figure 1. Comparison of total glycan signal intensities obtained using three different purification methods: **Cellulose (Sigma, Germany), Glycan-SPE (Sigma, Germany), and Mag-HILIC (BIO-DESIGN, Türkiye).** Bio-MagHILIC yielded the highest overall signal intensity, indicating superior glycan recovery efficiency compared to cellulose and Glycan-SPE approaches. Error bars represent standard deviations across replicates, highlighting the reproducibility of each method.

This bar chart compares the **total glycan signal (Total Height)** obtained from three different purification approaches:

- **Cellulose (Sigma, Germany)**
- **Glycan-SPE (Sigma, Germany)**
- **Mag-HILIC (BIO-DESIGN, Türkiye)**

The results clearly indicate that **BIO-DESIGN's Mag-HILIC shows the highest total glycan signal intensity**, reaching values close to 7.0×10^7, compared to ~4.5×10^7 for Cellulose and ~5.0×10^7 for Glycan-SPE. Error bars show the variability across replicates, highlighting that Mag-HILIC not only achieves higher recovery but also demonstrates consistent performance.

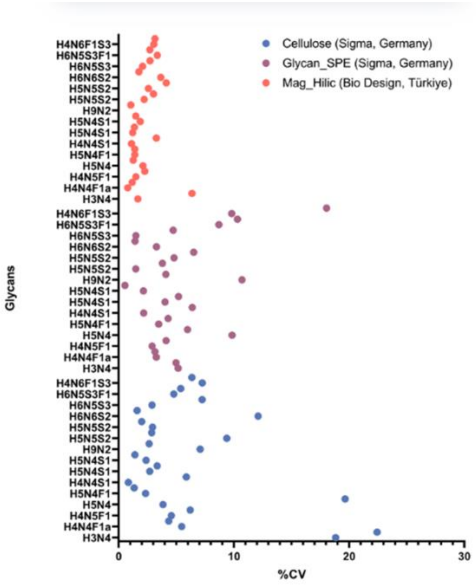


Figure 2. Coefficient of variation (%CV) values for individual glycans purified using **Cellulose (Sigma, Germany), Glycan-SPE (Sigma, Germany), and Mag-HILIC (BIO-DESIGN, Türkiye).** Bio-MagHILIC consistently yielded the lowest %CV across glycans, indicating superior reproducibility compared to cellulose and Glycan-SPE methods. Each dot represents a glycan species, with lower %CV values reflecting more robust quantification.

This scatter plot compares the **coefficient of variation (%CV)** for glycan measurements obtained with three different purification methods:

- **Cellulose (Sigma, Germany)** – blue
- **Glycan-SPE (Sigma, Germany)** – purple
- **Mag-HILIC (BIO-DESIGN, Türkiye)** – red

Interpretation

- **Lower %CV values represent higher reproducibility.**
- The plot shows that **Mag-HILIC (BIO-DESIGN, red)** consistently provides the lowest %CV across a wide range of glycans, clustering tightly near the 0–5% range.
- **Glycan-SPE (purple)** demonstrates moderate reproducibility, with %CV values generally below 10% but more scattered than Mag-HILIC.
- **Cellulose (blue)** exhibits the highest variability, with %CV values spreading broadly, in some cases exceeding 20–25%, indicating poor reproducibility compared to the other methods.

The figure demonstrates that **BIO-DESIGN's Mag-HILIC outperforms both Cellulose and Glycan-SPE methods in terms of reproducibility**. Its lower %CV values indicate more consistent and reliable glycan quantification, which is critical for applications in glycomics research and biopharmaceutical quality control.

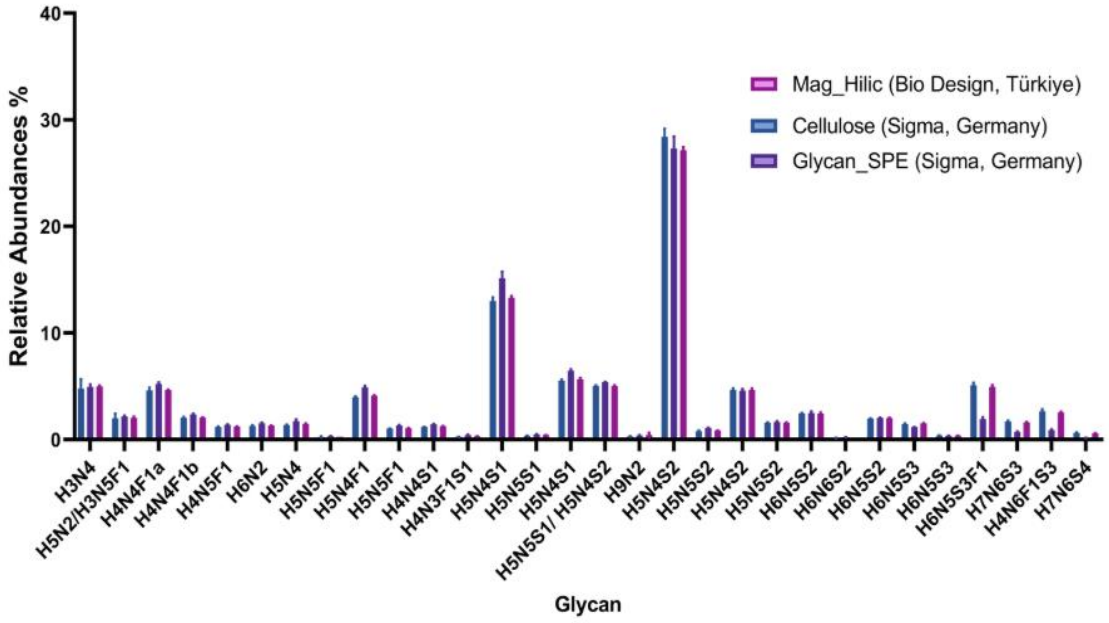


Figure 3. Relative abundances (%) of individual human serum N-glycans purified using **Mag-HILIC (BIO-DESIGN, Türkiye), Cellulose (Sigma, Germany), and Glycan-SPE (Sigma, Germany).** All three methods show comparable glycan distribution profiles, with Mag-HILIC demonstrating consistent coverage across both high- and low-abundance species. The data confirm that Bio-MagHILIC achieves equivalent structural resolution to established cellulose and SPE approaches, while offering improved reproducibility and handling efficiency.

This bar chart compares the **relative abundances (%) of individual N-glycan structures** purified from human serum using three different approaches:

- **Mag-HILIC (BIO-DESIGN, Türkiye)** – pink
- **Cellulose (Sigma, Germany)** – blue
- **Glycan-SPE (Sigma, Germany)** – purple

This figure demonstrates that **Bio-MagHILIC provides glycan profiles highly comparable to conventional cellulose and Glycan-SPE methods**, while maintaining robust detection of both major and minor glycan species. This confirms its suitability for **comprehensive and reproducible N-glycan profiling in complex biological samples such as human serum**.