

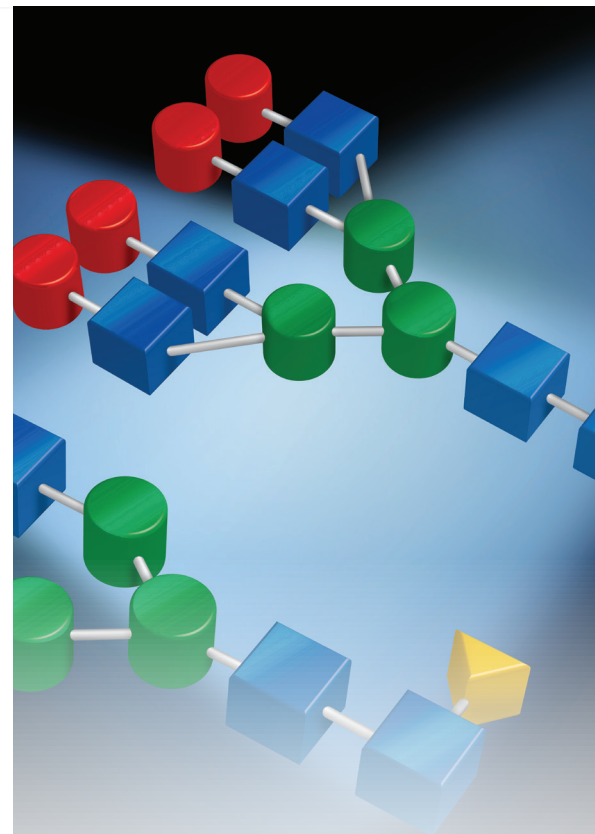
GlycoWorks Sample Preparation Consumables

Glycosylation is one of the most common forms of post-translational modification (PTM) of human and other eukaryotic proteins. Glycosylated proteins (glycoproteins) make up 50–70% of human proteins. Consequently, glycans play a critical role in a myriad of physiological and pathological reactions ranging from immunity, to blood clotting, to cell development, and cell death.

Glycoprotein analysis involves identifying complex N- and O-linked structures composed of frequently similar and repeating sugar moieties. Hydrophilic-interaction liquid chromatography (HILIC) with fluorescence detection is a well-recognized and reliable technique that effectively separates and quantitates isolated glycans after their derivatization with fluorescent labels.

Waters now offers the sample preparation aspect of the Glycan Application Solution to help users get the correct answers faster and more reliably:

- Versatile offering for individual samples up to high-throughput analyses
- Convenient, easy to use methodologies that bring a user through sample preparation to data collection and interpretation
- Quantitative and consistent recoveries for a diverse range of N-glycans with optimized SPE-conditions
- Support materials that range from inclusive care and use manuals to online training videos
- Seamless integration with Waters ACQUITY UPLC® and Alliance® HPLC platforms, related guard and column chemistries, and informatics solutions for data analysis.



SAMPLE PREPARATION

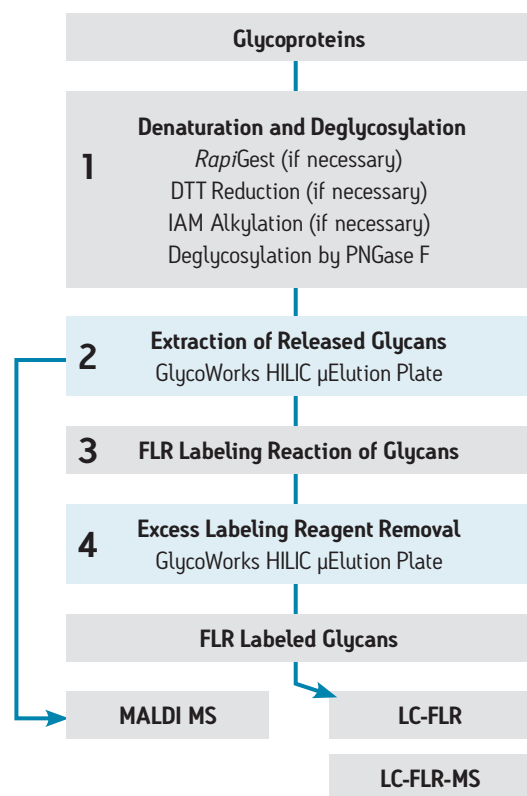
GlycoWorks Sample Preparation Kits

Waters GlycoWorks Consumables offer a more convenient, versatile, comprehensive and effective sample preparation solution for glycan analysis.

- Versatile formats for high throughput and single use device users
- *RapiGest*[™] for solubilization of the glycoprotein and efficient PNGase F enzymatic digestion of the N-linked glycans
- GlycoWorks Reagent Kit that contains compounds to make it easy for customers to follow the protocol
- Optimized HILIC Sorbent for high recovery of N-glycans and for effective labeling reaction clean up prior to LC/FLR/MS analysis



General Guideline of Sample Preparation from Glycoprotein to Enrich FLR Labeled Glycans Using Reductive Amination Reaction



GLYCOWORKS ADVANTAGES

Quantitative and consistent recoveries for a diverse range of N-glycans with optimized SPE-conditions

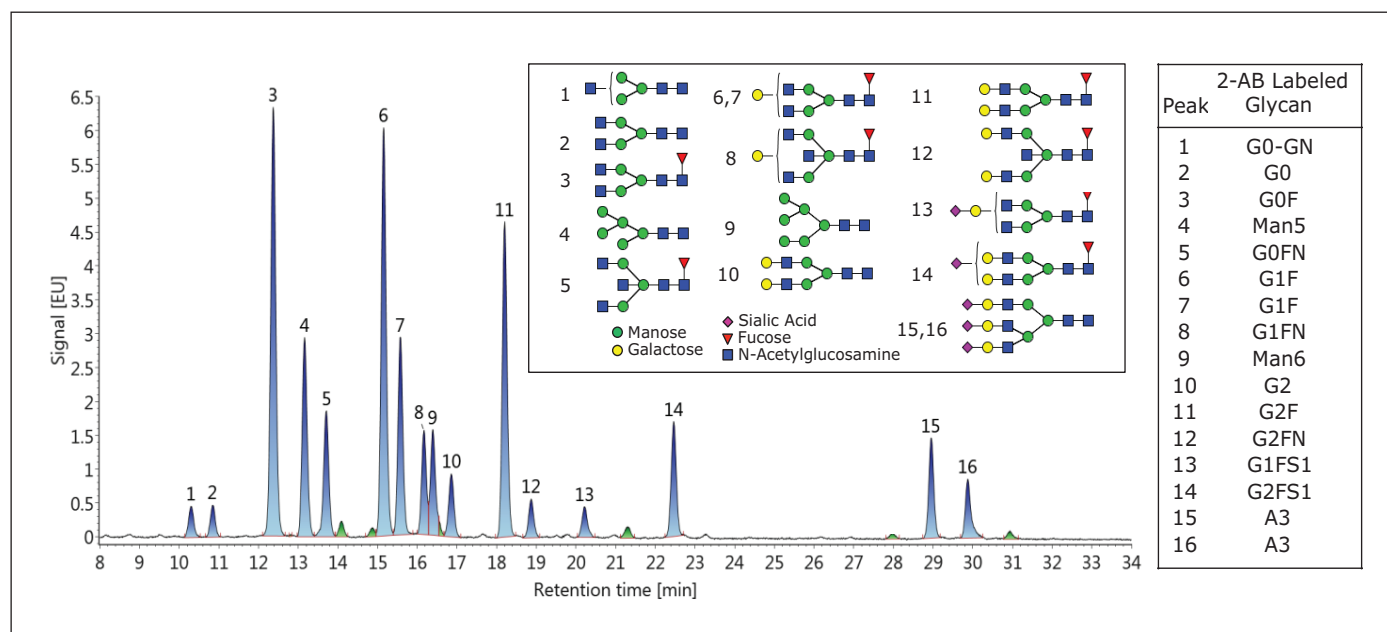


Figure 2. HILIC-FLR analysis of 2-AB labeled glycan performance test standard and trisialylated A3 glycans. 3 pmol of sample injected in 2.5 μL onto an ACQUITY UPLC GST Amide (BEH Glycan), 1.7 μm, 2.1 x 150 mm Column. Peaks detected by UNIFI processing are shaded in blue (expected component) or green (discovered component).

Excellent Method Robustness

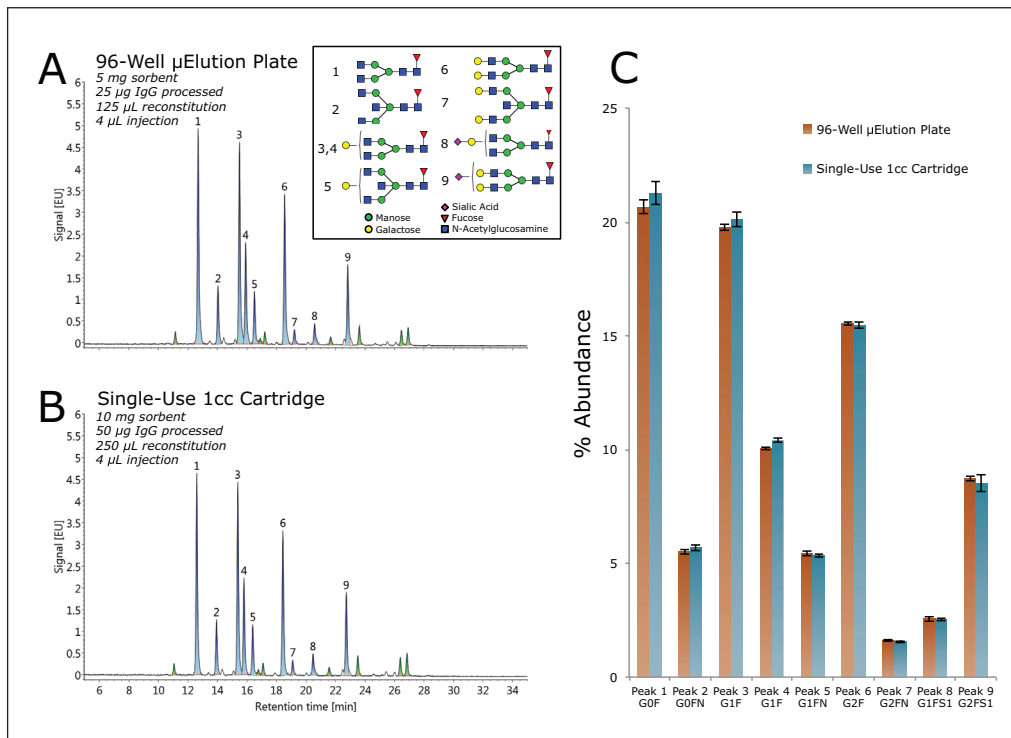


Figure 3. HILIC-FLR Analysis of 2-AB Labeled Glycans released from the GlycoWorks Control Standard using an ACQUITY UPLC GST Amide (BEH Glycan), 1.7 μm, 2.1 x 150 mm Column. The left panel displays chromatograms obtained for samples processed using (A) GlycoWorks HILIC 96-well μElution Plate, and (B) a single-use HILIC 1-cc cartridge. Peaks detected by UNIFI processing are shaded in blue (expected component) or green (discovered component). The integrated peak areas were used in the calculation of the relative abundances displayed on the right (C) (n=3).

ADDITIONAL CONSUMABLES

Dextran Calibration Ladder

The 2AB labeled Dextran Calibration Ladder allows the user to tie in the entire GlycoWorks Sample Preparation Solution seamlessly to the Waters ACQUITY UPLC System and GlycoBase Database Search. Using this standard allows the user to calibrate their system based on Glucose Units (GU) and have confidence in results.

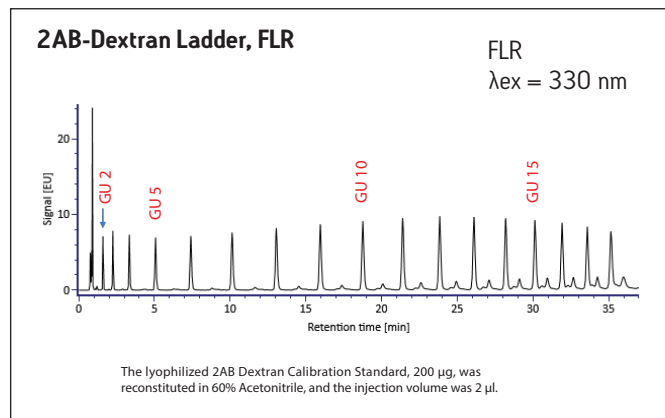


Figure 4: Example Chromatogram of the Dextran Calibration Ladder with GU Labeled Peaks. See Care and Use for more information.

Glycan Performance Test Standard

The Glycan Performance Test Standard is 2AB labeled human-like IgG and is QC verified to contain the components needed to benchmark and evaluate ACQUITY UPLC BEH Glycan, 1.7 μm Columns. It is also valuable to use as an additional 2AB labeled control to assess digestion and labeling reaction efficiencies.

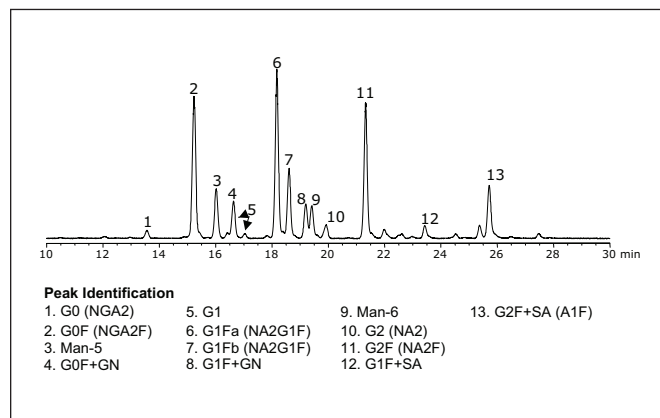


Figure 5: UPLC FLR Chromatogram of 2AB labeled Glycan Performance Test Standard. See Care and Use for more information.

Waters Positive Pressure-96 Processor

The Waters Positive Pressure-96 Processor offers state-of-the-art operation for 96-well plates and 1 cc flangeless cartridge formats. Each of the 96 holes in the processor is restricted in order to maintain constant pressure, even if all the plate well positions are not filled. Positive pressure processing offers many advantages over traditional methods, including:

- Highly uniform flow from well to well
- Superior flow for viscous samples
- Highly reproducible assays
- Easy-to-use design



UPLC AND HPLC BEH GLYCAN COLUMN OFFERINGS

Waters BEH-based Glycan chemistry offerings are available in three highly scalable particle sizes that address UPLC® (i.e., 1.7 µm) and HPLC-based (2.5 µm *XP* and 3.5 µm) application needs. Each batch of BEH Glycan material is specifically quality control tested with the 2AB labeled, Waters Glycan Performance Standard to help ensure batch-to-batch consistency as well as highly similar separated glycan profiles to help ensure highly similar. Consequently, chromatographers can now choose the most appropriate LC-based technology to address their specific released glycan analysis application needs and laboratory instrumentation.

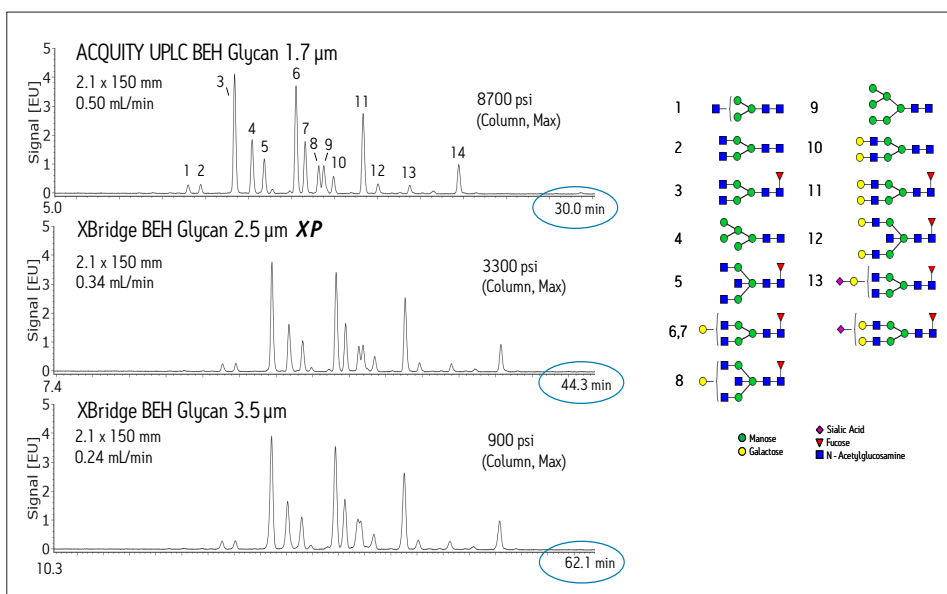
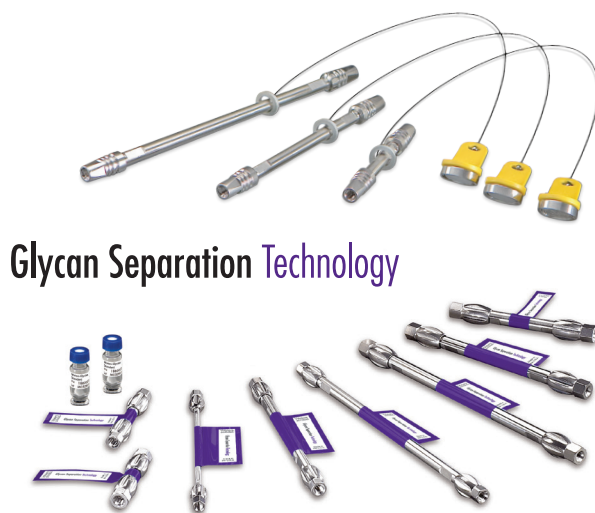


Figure 6. Column lengths that satisfy resolution and sample throughput requirements.

GLYCAN ANALYSIS WORKFLOW

Sample Preparation:

GlycoWorks line of sample preparation products and additional support consumable standards

- Quantitative and consistent recoveries
- Excellent method robustness
- Relevant support standards

Chromatographic Analysis:

ACQUITY UPLC System with FLR detection and ACQUITY UPLC BEH Glycan Columns

- Improved component resolution in less time compared to existing HPLC-based methods
- Quantitative information provided for a range of glycans

Data Analysis and Interpretation:

Coupled with Empower® or UNIFI® to acquire and process data.

- Utilize the Waters' UPLC Database within NIBRT's GlycoBase 3.0
- This UPLC database was developed by the Waters-NIBRT collaboration

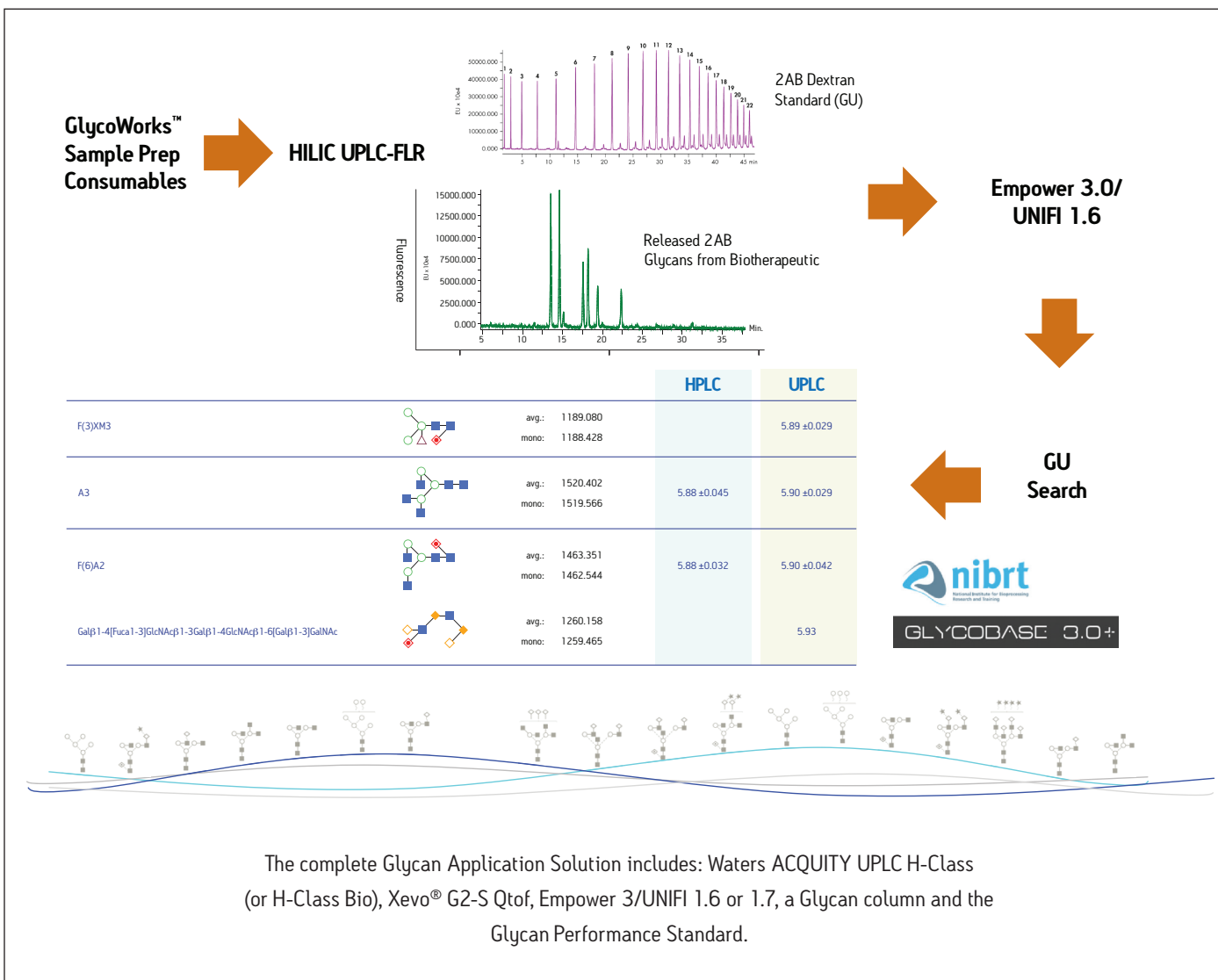


Figure 7. Waters glycan analysis work flow

VI. ORDERING INFORMATION

Glyco Sample Preparation Kit and Standards

Description	Part No.
GlycoWorks High-throughput Prep Kit	176003090
GlycoWorks HILIC μ Elution Plate, 96-well	186002780
RapiGest SF 1 mg Vial	186001860
GlycoWorks Control Standard, 100 μ g Vial	186007033
GlycoWorks Reagent Kit	186007034
Manifold Waste Tray	600001282
GlycoWorks Single Use Prep Kit	176003119
GlycoWorks HILIC 1 cc Cartridge (20/pk)	186007080
RapiGest SF 1 mg Vial	186001860
GlycoWorks Control Standard, 100 μ g Vial	186007033
GlycoWorks Reagent Kit	186007034
Glycan Performance Test Standard	186006349
The Glycan Performance Test Standard is a 2-AB labeled human IgG-like standard that is QC verified to contain the components needed to benchmark and evaluate ACQUITY UPLC BEH Glycan, 1.7 μ m Columns.	
Dextran Calibration Ladder	186006841
The 2-AB labeled, Dextran Calibration Ladder is used to calibrate the HILIC column from retention time to GU values. This calibration ladder provides good peak shape and reliable identification from 2 to 30 Glucose Units.	
GlycoWorks HILIC 1 cc Flangeless Cartridges Pack	186007239
This pack of 20 cartridges, good for 10 analyses, are offered for those customers who prefer to use the single use devices with the positive pressure manifold (PPM).	

Glycan Analysis System Standards and Mobile Phases

Description	Usage	Volume	Part No.
Alliance with Fluorescence Qualification Standards Test Kit	C P		700002753
Kit contains seven 10 mL vials of varying concentrations of anthracene in 80:20 acetonitrile/water:			
(1) 0.5 pg/ μ L (1) 10.0 pg/ μ L	(1) 1.0 pg/ μ L (1) 2.5 ng/mL	(2) 5.0 pg/ μ L (1) 2.5 μ g/mL	
One blank 10 mL vial of 80:20 acetonitrile/H ₂ O			
Fluorescence Detector Standard Solution	P		700003694
5.0 pg/ μ L anthracene in 20:80 water/acetonitrile		1 mL	
Fluorescence Detector Performance Standard Solution	P		WAT047685
0.10 mg/L anthracene in 70:30 acetonitrile/water		10 mL	
Ammonium Formate Concentrate			186007081
5000 mM Ammonium Formate in water with 3.8% Formic acid matrix. This allows the end user to dilute the concentrate 10 mL to 1 L before using to reach a 50 mM concentration.		10 mL	

C Calibration **P** Performance Check

Additional Consumables

Description	Part No.
GlycoWorks HILIC 1 cc Flangeless Cartridges Pack	186007239
Dextran Calibration Ladder	186006841
Glycan Performance Test Standard	186006349
RapiGest SF	186001861
96-well Collection Plate	186002481
Cap Mats for 1 mL Collection Plate	186002483
μ Elution Plate Manifold	186001831
SPE Vacuum Pump	176002986
Positive Pressure Manifold	186006961

Glycan Separation Technology Columns

Description	Particle Size	Inner Diameter	Length	Part Number
XBridge BEH Glycan XP Column	2.5 µm	2.1 mm	50 mm	186007263
XBridge BEH Glycan XP Column	2.5 µm	2.1 mm	100 mm	186007264
XBridge BEH Glycan XP Column	2.5 µm	2.1 mm	150 mm	186007265
XBridge BEH Glycan XP Column	2.5 µm	4.6 mm	50 mm	186007268
XBridge BEH Glycan XP Column	2.5 µm	4.6 mm	100 mm	186007269
XBridge BEH Glycan XP Column	2.5 µm	4.6 mm	150 mm	186007270
XBridge BEH Glycan Column	3.5 µm	4.6 mm	50 mm	186007273
XBridge BEH Glycan Column	3.5 µm	4.6 mm	100 mm	186007274
XBridge BEH Glycan Column	3.5 µm	4.6 mm	150 mm	186007275
XBridge BEH Glycan Column	3.5 µm	4.6 mm	250 mm	186007276
XBridge BEH Glycan VanGuard Pre-column, 3/pkg	2.5 µm	2.1 mm	5 mm	186007262
XBridge BEH Glycan Sentry Guard, 2/pkg	2.5 µm	4.6 mm	20 mm	186007267¹
XBridge BEH Glycan Sentry Guard, 2/pkg	3.5 µm	4.6 mm	20 mm	186007272¹
XBridge BEH Glycan Method Validation Kit, 3/pkg	2.5 µm	2.1 mm	150 mm	186007266
XBridge BEH Glycan Method Validation Kit, 3/pkg	2.5 µm	4.6 mm	150 mm	186007271
XBridge BEH Glycan Method Validation Kit, 3/pkg	3.5 µm	4.6 mm	150 mm	186007277
XBridge BEH Glycan Column	3.5 µm	2.1 mm	50 mm	186007502
XBridge BEH Glycan Column	3.5 µm	2.1 mm	100 mm	186007503
XBridge BEH Glycan Column	3.5 µm	2.1 mm	150 mm	186007504
XBridge BEH Glycan Sentry Guard, 2/pkg	3.5 µm	2.1 mm	10 mm	186007505²

¹ Requires 2.1 x 10 mm Universal Sentry Guard Holder, Part No. WAT097958.

² Requires 4.6 x 20 mm Universal Sentry Guard Holder, Part No. WAT046910.

References

1. Lauber MA, Koza S, Fountain KJ. Optimization of HILIC SPE for the Quantitative and Robust Recovery of N-Linked Glycans. Waters Application Note [720004710EN](#). 2013 June.
2. Lauber MA, Koza S, Fountain KJ. Single-Use and High-Throughput HILIC SPE Device Formats and an IgG Control Standard for Facilitating N-Glycan Analyses Waters Technology Brief [720004711EN](#). 2013 June.
3. Training Video: http://www.waters.com/waters/en_US/Glycan-Standards/nav.htm?cid=134640534
4. GlycoWorks Sample Preparation Protocol for MALDI. Waters Application Note [720004660en](#)

Waters

THE SCIENCE OF WHAT'S POSSIBLE.®

Waters, The Science of What's Possible, Xevo, ACQUITY UPLC, UPLC, Alliance, Empower, and UNIFI are registered trademarks of Waters Corporation. GlycoWorks, RapiGest, and VanGuard are trademarks of Waters Corporation. All other trademarks are the property of their respective owners.

©2013 Waters Corporation. Produced in the U.S.A.
December 2013 720004584EN SC-PDF

Waters Corporation
34 Maple Street
Milford, MA 01757 U.S.A.
T: 1 508 478 2000
F: 1 508 872 1990
www.waters.com

