

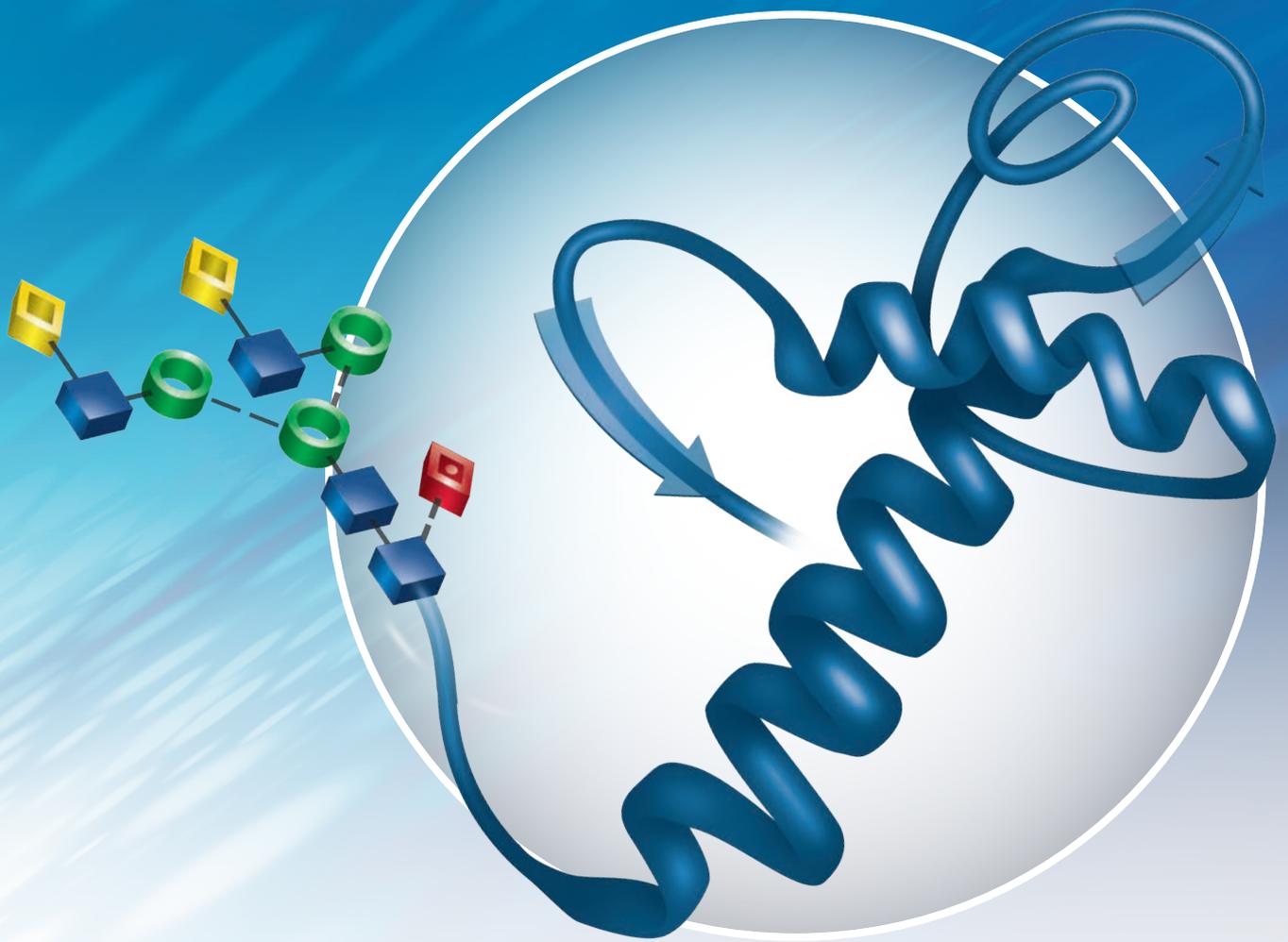
[GLYCAN ANALYSIS SOLUTIONS]



The **Next Generation**
of Glycan Sample Preparation
and Analysis

Waters

THE SCIENCE OF WHAT'S POSSIBLE.®



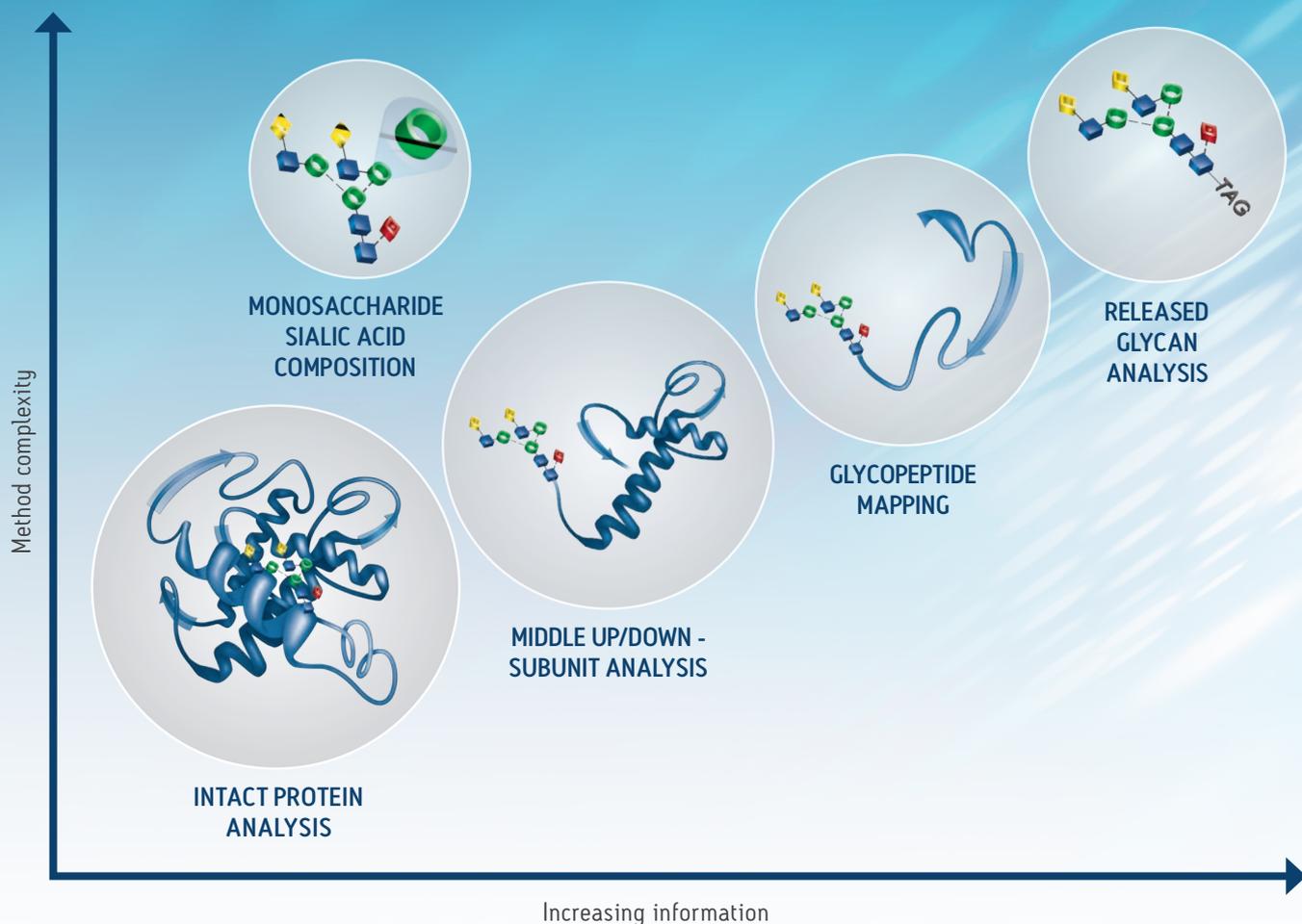
What if...

identifying **WHERE** glycans are present on a glycoprotein becomes part of your routine workflow?

Bringing Together Complementary Techniques to Streamline Glycan Analysis

Waters offers complete, workflow-based approaches for analyzing glycoproteins at all structural levels.

- Glycoprotein profiling
- Middle up/down - Subunit analysis
- Glycopeptide mapping
- Released glycan characterization and monitoring
- Monosaccharide/Sialic acid composition





Intact Protein Analysis Workflow

GLYCOPROTEIN

Glycoprotein Performance Test Standard



INJECTION ON COLUMN FOR LC ANALYSIS
ACQUITY UPLC® Glycoprotein BEH Amide, 300Å Column, and MassPREP™ Desalting Cartridge



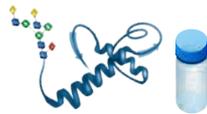
ANALYSIS ON LC WITH TUV OR FLR DETECTOR
ACQUITY UPLC Systems



Subunit Analysis Workflow

GLYCOPROTEIN

Intact mAb Mass Check Standard



IdeS ENZYMATIC DIGESTION WITH OPTIONAL REDUCTION



INJECTION ON COLUMN FOR LC ANALYSIS
ACQUITY UPLC Glycoprotein BEH Amide, 300Å Column, and Glycoprotein Performance Test Standard



ANALYSIS ON LC WITH UV DETECTOR
ACQUITY UPLC Systems



Glycopeptide Mapping Workflow

GLYCOPROTEIN

Intact mAb Mass Check Standard



REDUCTION, ALKYLATION, AND ENZYME DIGESTION(S)

Glycosylated and non-glycosylated peptide fragments



INJECTION ON COLUMN FOR LC ANALYSIS
ACQUITY UPLC Glycoprotein BEH Amide, 300Å Column, and Glycoprotein Performance Test Standard



ANALYSIS ON LC WITH TUV DETECTOR
ACQUITY UPLC Systems



Monosaccharide/Sialic Acid Composition Workflow

GLYCOPROTEIN

Intact mAb Mass Check Standard



RELEASE OF MONOSACCHARIDES
Hydrolyze with acid



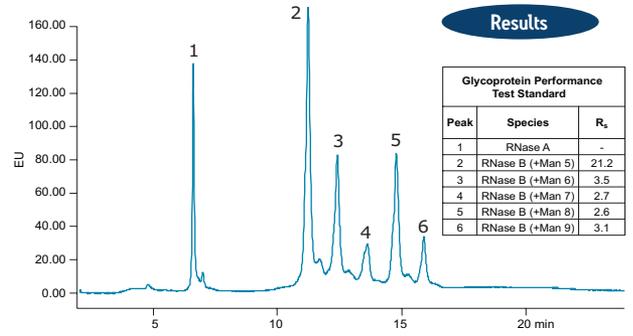
LABEL WITH A FLUOROPHORE



Injection on Column for LC Analysis
ACQUITY UPLC BEH C₁₈, 130Å, 1.7 µm, 2.1 x 100 mm Column

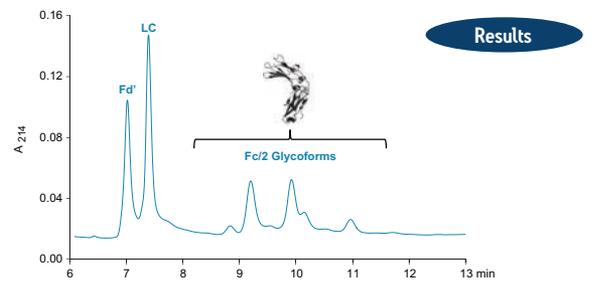
→ **MASS CONFIRMATION WITH QToF MS**
 Xevo® G2-XS QToF MS, and SYNAPT® G2-Si HDMS

→ **DATA PROCESSING**
 UNIFI®, and MassLynx® Software



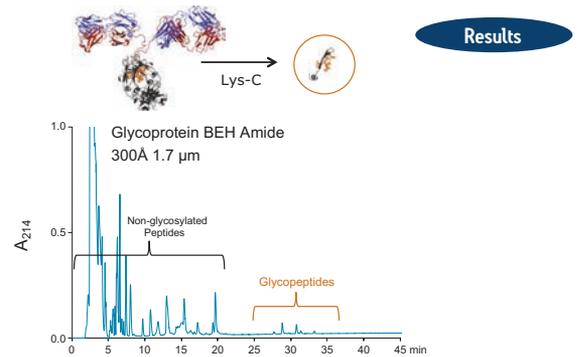
→ **MASS CONFIRMATION USING QToF MS**
 Xevo G2-XS QToF MS, and SYNAPT G2-Si HDMS

→ **DATA PROCESSING**
 MassLynx Software



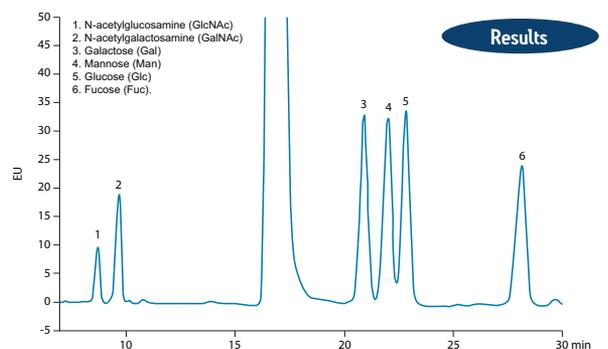
→ **PEPTIDE MAPPING ANALYSIS USING QToF MS**
 Xevo G2-XS QToF, and SYNAPT G2-Si HDMS

→ **DATA PROCESSING**
 MassLynx, and UNIFI Software



→ **Analysis on LC with FLR Detector**
 Alliance HPLC or ACQUITY UPLC Systems

→ **DATA PROCESSING**
 Empower® 3 Software



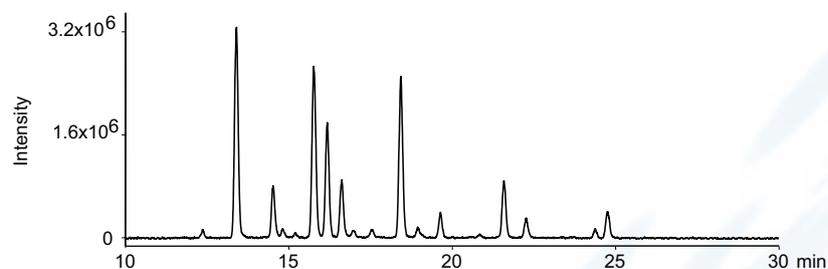
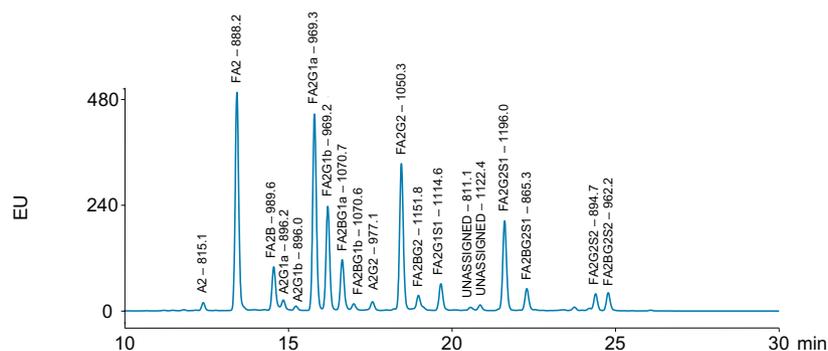
ROUTINE MONITORING

Explore the possibilities

Due to the increased ionization efficiency and higher proton affinity of *RapiFluor*-MS, mass detection of glycans using the ACQUITY QDa Detector is finally possible.

- Provides unrivaled sensitivity for FLR and MS detection.
- Offers greater confidence with mass confirmation of FLR peaks.
- Reduce chromatography analysis times with targeted MS glycan profiling for rapid process development.
- Can be easily integrated with existing GMP-compliant-ready UPLC workflows with Empower 3 Software.

Glycan Monitoring with ACQUITY UPLC H-Class Bio System with FLR and ACQUITY QDa Detection



RapiFluor-MS labeled N-glycans monitored using an ACQUITY UPLC H-Class Bio System configured with FLR and ACQUITY QDa mass detection for confirmation of known structures and mass data for unassigned peaks.

Introducing the ACQUITY UPLC Glycoprotein BEH Amide, 300Å, 1.7 μm Column

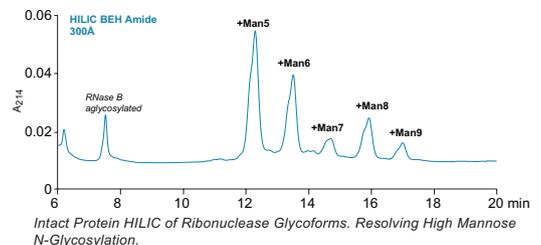
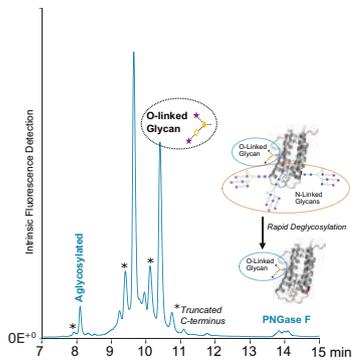
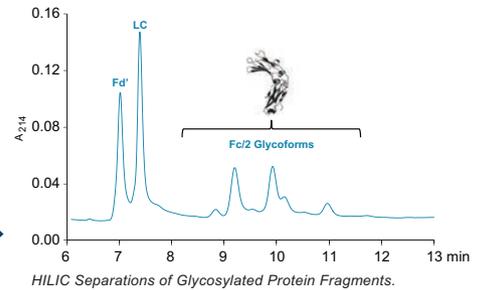
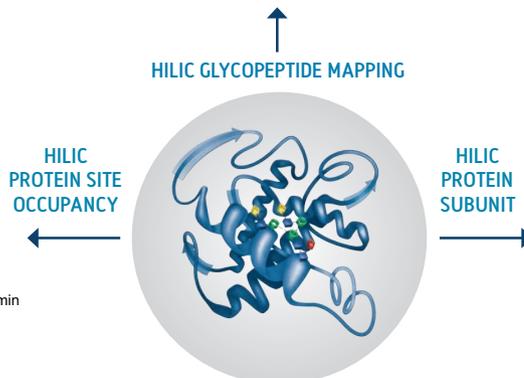
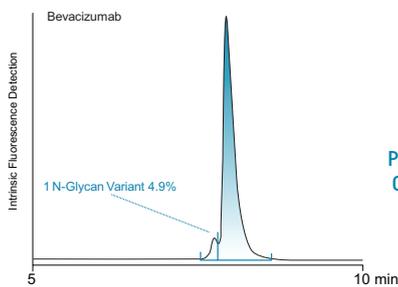
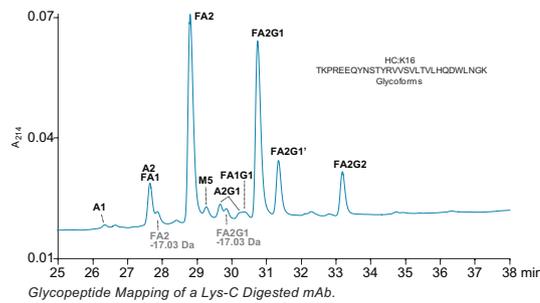
You need to know **WHAT** your glycan structures are, **WHY** not also know **WHERE** they are...

With Waters, now you can combine approaches for comprehensive characterization. The ACQUITY UPLC® Glycoprotein BEH Amide 300Å Column's wide pore stationary phase and optimized amide bonding, provides unprecedented, MS compatible HILIC-based separations for the analysis of intact glycoproteins, glycoprotein fragments, and glycopeptides.

This new, patent pending column technology allows access to information about:

- Site-specific glycan structures
- Glycan occupancy

One column that brings separation opportunities never seen before...





What if...

released N-glycan analysis could be
both streamlined **and** highly sensitive?

Finally, a smart workflow with no compromises.

Introducing the GlycoWorks *RapiFluor*-MS N-Glycan Kit

Waters, the leader in innovations for biopharmaceutical analysis, has reimagined the workflow for released N-glycan analysis. Our new reagent, *RapiFluor*-MS™ features the ionization capability necessary to maximize the information generated by MS detection. Our new workflow speeds up deglycosylation and streamlines labeling.

It's the smart workflow that biopharmaceutical laboratories have been waiting for—you no longer have to choose between a “quick” labeling tool and sensitivity for mass spectrometry; no more labor-intensive and complicated processes that leave room for error.

With Waters, there are no compromises between speed and sensitivity in sample preparation—only gains in confidence in your analytical results.



Join Waters in thinking outside the box
for released glycan analysis.

SPEED/THROUGHPUT



FLR QUANTIFICATION



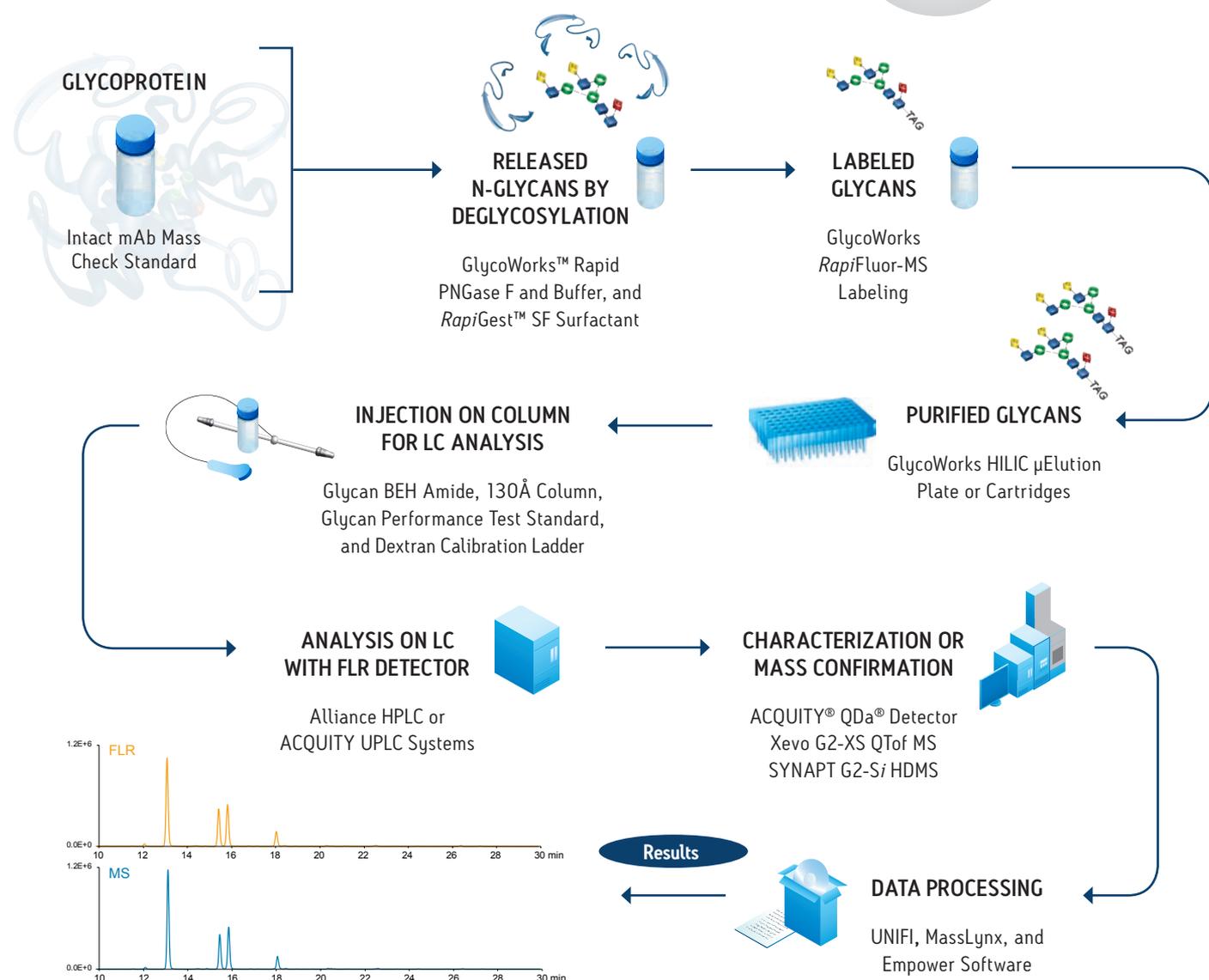
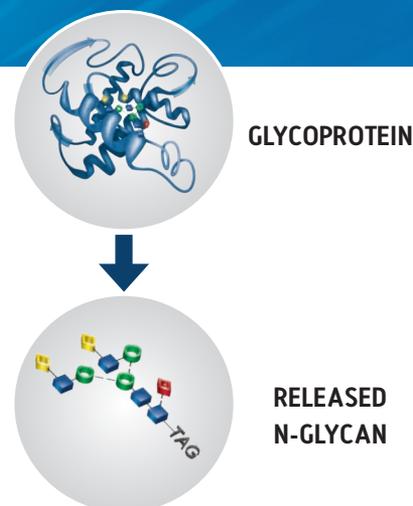
MS PERFORMANCE



How does it work?

Released N-glycan analysis is an information-rich technique, but it has involved complicated sample preparation procedures. Once glycans are released by PNGase F, labeling is required for effective separation and detection. Using Waters' new protocol, you now have a versatile workflow for:

- Glycan identification
- Glycan structure
- Glycan monitoring

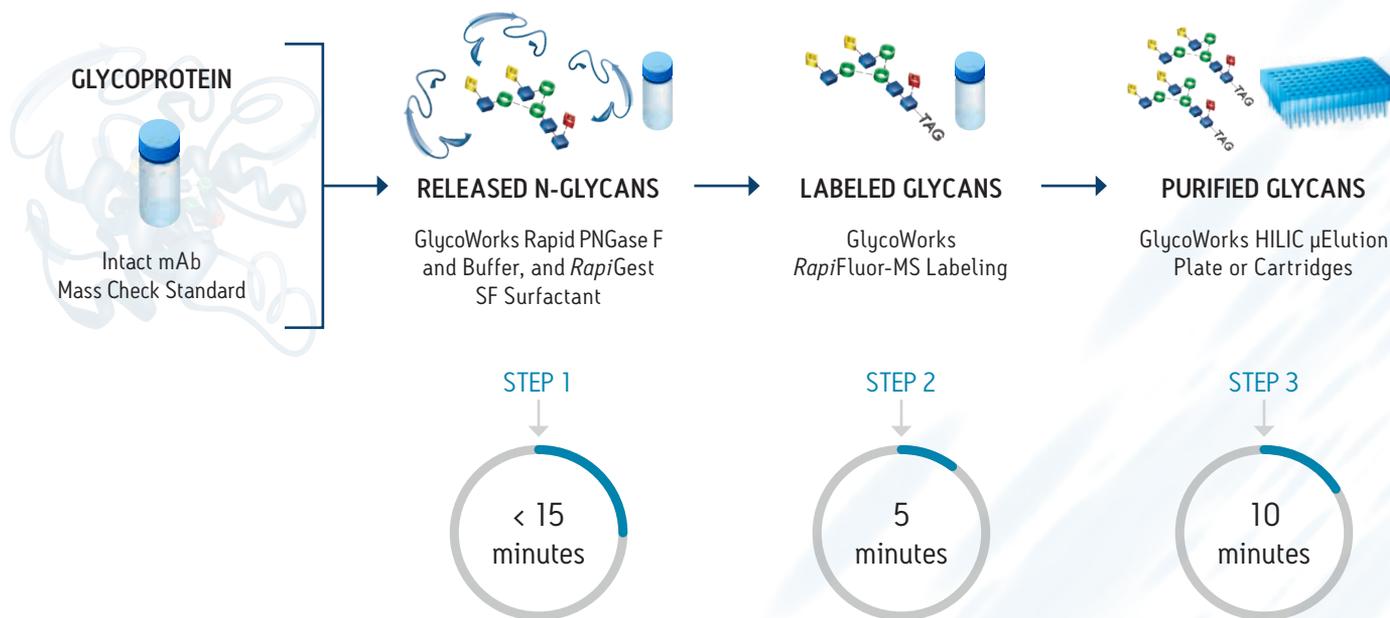


See the science behind our **NEW GlycoWorks RapiFluor-MS Reagent**, which enables new capabilities for glycan analysis, monitoring and characterization. Watch our video at waters.com/SeeTheScience

What if released N-glycan sample preparation was fast and simplified?

- Easily and quickly prepare labeled released N-glycan samples.
- Minimize error and sample loss.
- Easily train analysts and transfer methods throughout your organization.

3 STEPS, 30 MINUTES



Watch a short 2 minute RapiFluor-MS
N-glycan Kit demonstration video.

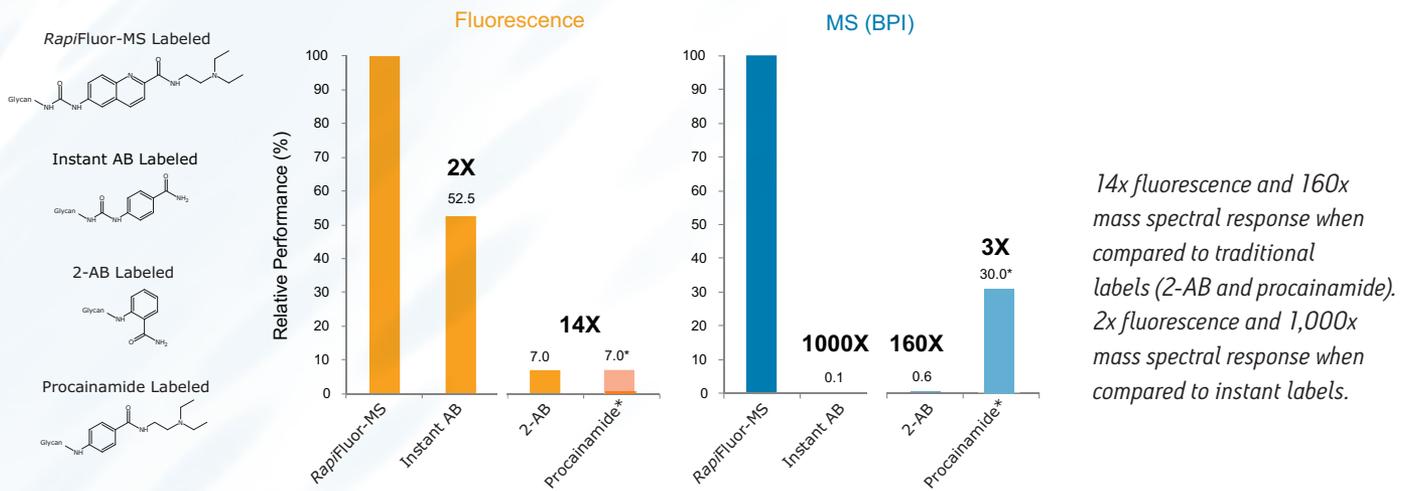
www.waters.com/RapiFluorMS



What if you could obtain unrivaled FLR and MS sensitivity?

- Improve product quality understanding with in-depth characterization of previously unidentified peaks with fluorescence quantification obtained with equivalent mass spectral response.
- Accelerate decision making.
- More easily identify low abundance N-linked glycans.

UNRIVALED SENSITIVITY



*Based on published theoretical values.

See how you can bring extraordinary possibilities to your everyday analysis.

www.waters.com/XevoG2XS

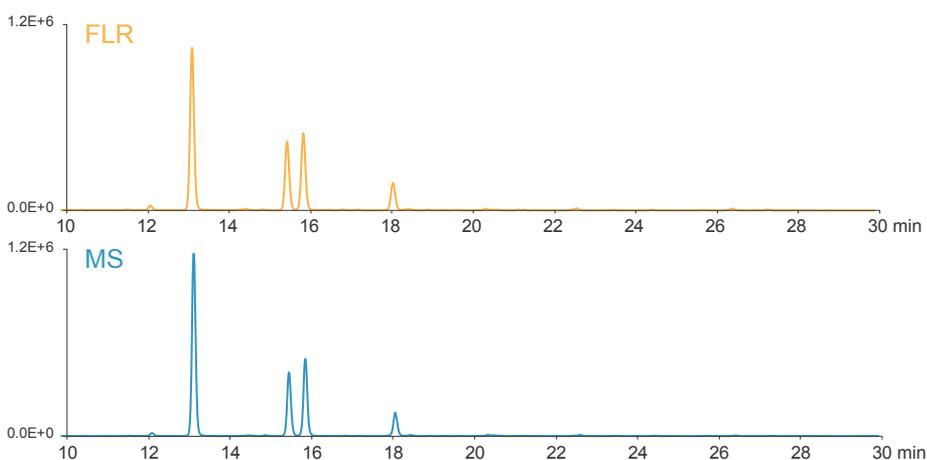


What if mass data was easily and always available for routine glycan analysis?

- Increase confidence in glycan profile monitoring with routine mass detection.
- Obtain information about manufacturing changes with quick sample turnaround.
- Resolve method development and transfer issues quickly.

CONFIDENCE

Detailing the N-glycan Profile of a mAb



The increased ionization efficiency of RapiFluor-MS allows for quantitative fluorescence paired with mass detection with the ACQUITY QDa Detector.

Watch a short 1 minute video on how you can bring the power of mass detection to your lab.

www.waters.com/QDa



Ordering Information

Released N-Glycan Sample Preparation Complete Kits

Description	Part No.
GlycoWorks RapiFluor-MS N-Glycan Starter Kit—96 Sample Kit contains: GlycoWorks Deglycosylation Module, GlycoWorks Labeling Module, GlycoWorks Clean-up Module, GlycoWorks Sample Collection Module, ACQUITY UPLC Glycan BEH Amide, 1.7 µm, 2.1 x 150 Column, Mobile Phase Concentrate: Ammonium Formate	176003635
GlycoWorks RapiFluor-MS N-Glycan Kit—96 Sample Kit contains: GlycoWorks Deglycosylation Module, GlycoWorks Labeling Module, GlycoWorks Clean-up Module, GlycoWorks Sample Collection Module	176003606
GlycoWorks RapiFluor-MS N-Glycan Starter Kit—24 sample Kit contains: GlycoWorks Deglycosylation Module (24 sample), GlycoWorks Labeling Module (24 sample), GlycoWorks Clean-up Module, GlycoWorks Sample Collection Module, ACQUITY UPLC Glycan BEH Amide, 1.7 µm, 2.1 x 150 mm Column, Ammonium Formate Solution – Glycan Analysis	176003712
GlycoWorks RapiFluor-MS N-Glycan Kit—24 sample Kit contains: GlycoWorks Deglycosylation Module (24 sample), GlycoWorks Labeling Module (24 sample), GlycoWorks Clean-up Module, GlycoWorks Sample Collection Module	176003713
GlycoWorks RapiFluor-MS N-Glycan Refill Kit—24 sample Refill Kit contains one of each: GlycoWorks Deglycosylation Module (25 sample) and the GlycoWorks Labeling Module (24 sample)	176003714

Released N-Glycan Standards and Accessories

Description	Part No.	Description	Part No.
Vacuum Manifold Shims*, 3/set	186007986	96-Well Plate Extraction Manifold	186001831
Positive Pressure Manifold Spacer for the GlycoWorks RapiFluor-MS N-Glycan Kit*, 1/pack	186007987	Vacuum Pump 220 v/240 v 50 Hz	725000604
RapiFluor-MS Dextran Calibration Ladder, 50 µg/vial	186007982	Positive Pressure Manifold	186006961
RapiFluor-MS Glycan Performance Test Standard, 400 pmol total/vial	186007983	Modular Heat Block for 1 mL Tubes, 1 mL tubes/96 wells	186007985
RapiGest SF, 10 mg vial	186002123	GlycoWorks Rapid Buffer, 5 mL	186008100
Ammonium Formate Solution – Glycan Analysis, 5000 mM	186007081	RapiGest SF, 3 mg vial	186008090
Intact mAb Mass Check Standard**	186006552	ACQUITY UPLC Glycan BEH Amide, 1.7 µm, 2.1 x 150 mm Column	186004742

*Essential for kit use

**Control Standard included in kit

Glycoprotein Columns and Standard

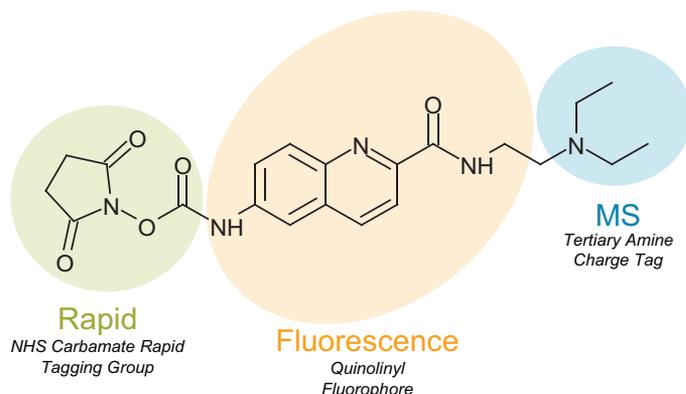
Description	Pore Size	Particle Size	Dimensions	Part No.
ACQUITY UPLC Glycoprotein BEH Amide and Std.	300Å	1.7 µm	2.1 x 5 mm Guard 3/pk with Std.	176003699
ACQUITY UPLC Glycoprotein BEH Amide and Std.	300Å	1.7 µm	2.1 x 50 mm with Std.	176003700
ACQUITY UPLC Glycoprotein BEH Amide and Std.	300Å	1.7 µm	2.1 x 100 mm with Std.	176003701
ACQUITY UPLC Glycoprotein BEH Amide and Std.	300Å	1.7 µm	2.1 x 150 mm with Std.	176003702
ACQUITY UPLC Glycoprotein BEH Amide and Std.	300Å	1.7 µm	2.1 x 100 MVK, 3/pk with Std.	176003703
Glycoprotein Performance Test Standard				186008010

The next generation of glycan labeling

Built upon Waters' expertise in rapid fluorescence labeling of amino acids, *RapiFluor-MS* is designed with enhanced chemical properties for superior glycan analysis. Within a 5-minute reaction, *RapiFluor-MS* modifies glycosylamines generated by enzymatic release from a glycoprotein.

- Rapid tagging
- Efficient fluorescence
- Unrivaled mass spectral response

RapiFluor-MS Reagent



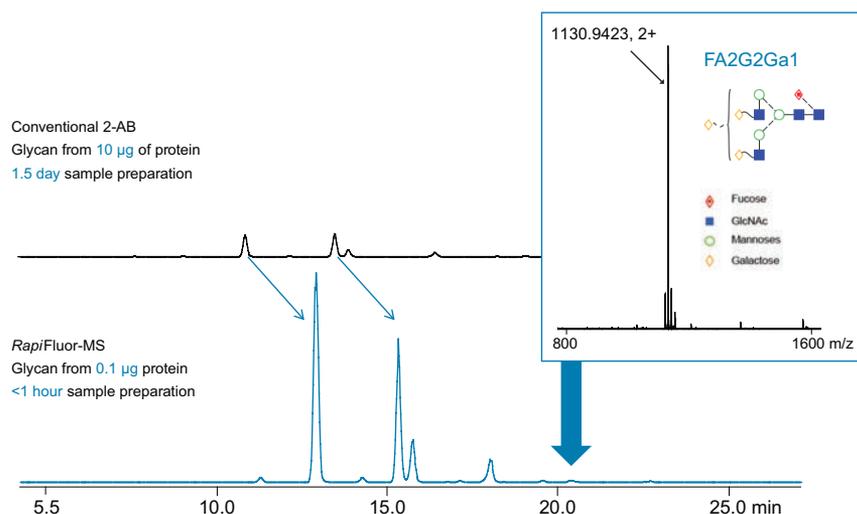
GlycoWorks *RapiFluor-MS*

CHARACTERIZATION

Assign low-level glycan components with more ease and confidence

Achieve unparalleled levels of MS and MS/MS sensitivity structural assignments by combining *RapiFluor-MS* with the best-in-class Xevo G2-XS QToF or SYNAPT G2-Si HDMS Mass Spectrometers.

Glycan Characterization by UPLC® FLR with Xevo G2-XS QToF Mass Spectrometer



Comparison of LC-MS chromatogram of 2AB-labeled *N*-glycans versus *RapiFluor-MS* labeled *N*-glycans from the same mAb. 100 fold greater ESI+ MS response was observed for *RapiFluor-MS* labeled sample. An MS spectrum (inset) shows the summed spectrum for a low abundance glycan within the sample.

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www.waters.com/glycans

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