

Application Note

Sample Clean-Up After N-Linked Glycan Release and Labeling Using bioZen™ N-Glycan Clean-Up HILIC Solid Phase Extraction (SPE) Microelution 96-Well Plate

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Overview

This application note compares two glycan clean-up solutions for a commercially available in-solution digestion and labeling kit for glycan profiling of glycoproteins. bioZen N-Glycan Clean-Up is a HILIC solid phase extraction product in a microelution 96-well plate that has excellent retention and recovery of labeled, released n-glycans. In this study, a trastuzumab biosimilar and a heavily sialylated glycoprotein (alpha-1 acid glycoprotein, AGP) were deglycosylated and glycans were labeled using the Waters® GlycoWorks™ RapiFluor-MS™ workflow. To remove excess dye, the samples were extracted using two different HILIC plates: bioZen N-Glycan Clean-Up microelution plate and the GlycoWorks HILIC μElution plate. For the trastuzumab biosimilar, the recovery of key labeled glycans, as determined by peak areas, were then compared. For AGP, a weighted average of charge (Z-value) was determined. The bioZen N-Glycan Clean-Up microelution plate gave comparable results to the GlycoWorks HILIC μElution plate, providing an alternative SPE product in the glycan workflow offered at a cost savings for labs running glycan clean-up and analysis.

Experimental Conditions

Waters GlycoWorks RapiFluor-MS N-glycan profiling kit was obtained from Waters (Waltham, MA, USA).

Deglycosylation and Labeling: Samples were reconstituted to 2 mg/mL in pure water. Deglycosylation and labeling were performed per vendor recommendations. Each sample was loaded in quadruplicate onto both glycan clean-up plates.

Solid Phase Extraction Protocol

- 96-Well Plate:** Waters GlycoWorks HILIC μElution Plate
bioZen N-Glycan Clean-Up Microelution Plate
- Part No.:** 8M-S009-NGA (bioZen)
- Condition:** 200 μL Water
- Equilibrate:** 200 μL Water/Acetonitrile (15:85)
- Load:** Labeled sample (~400 μL)
- Wash:** 2x 600 μL Formic acid/Water/Acetonitrile (1:9:90)
- Elute:** 3x 30 μL 200 mM Ammonium acetate in Acetonitrile/Water (5:95)

Each sample was analyzed using a generic amide LC column on a Waters ACQUITY® UPLC iClass equipped with fluorescence detection using standard methods and conditions (data not shown).

Results

The efficiency and peak areas of the released N-glycans from the trastuzumab biosimilar were compared between the bioZen N-Glycan Clean-Up microelution plate and the Waters GlycoWorks HILIC μElution Plate. G1 and G1F isomers were summed for simplicity and the data is shown in **Table 1**.

Figure 1.
Average Peak Areas of Labeled Glycans

Labeled Glycan	bioZen N-Glycan Clean-Up Average Peak Area	Waters GlycoWorks HILIC μElution Average Peak Area	% Difference
G0	132898	128611	103%
G0F	1131650	1082913	105%
G1/G1'	59236	56873	104%
G1F/G1F'	35168	332001	106%

Because AGP is composed mainly of multi-antennae, sialylated glycans, Z-values were used for comparison. Z-value takes a weighted average of contributing charge shares for different levels of sialylation and could reveal a bias or difference in recovery of different forms of sialylated glycans.

Figure 2.
Comparison of Z-value determination for AGP

Peak Group	bioZen N-Glycan Clean-Up Microelution Plate			Waters GlycoWorks μElution Plate		
	Percentage of peak group area (%)	Multiplication Factor	Charge Number Share	Percentage of peak group area (%)	Multiplication Factor	Charge Number Share
Asialo	0.3	0	0.0	0.4	0	0.0
Monosialo-	14.4	1	14.4	14.6	1	14.6
Disialo-	1.8	2	3.6	2.0	2	3.9
Trisialo-	57.6	3	172.7	57.8	3	173.3
Tetrasialo-	25.9	4	103.5	24.8	4	99.2
	Z=294.2			Z=291.0		

Conclusion

The bioZen N-Glycan Clean-Up microelution plate is an appropriate replacement for the Waters GlycoWorks μElution plates in the Waters GlycoWorks RapiFluor-MS glycan workflow solution.



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Ordering Information

Sample Preparation

bioZen™ Solid Phase Extraction	Format	Sorbent Mass	Part Number	Unit
bioZen N-Glycan Clean-Up	Microelution 96-Well Plate	5 mg/well	8M-S009-NGA	1/box

LC Columns

bioZen Columns (mm)	Biocompatible Guard Cartridges			
	100 x 2.1	150 x 2.1	for 2.1 mm	Holder
bioZen 2.6 µm Glycan	00D-4773-AN	00F-4773-AN	AJ0-9800	AJ0-9000



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