

EZGlyco® O-Glycan Prep Kit Application notes (Precautions for use)

When samples contain a large amount of low molecular weight excipients such as salts, stabilizers and sugars, the filter column may become clogged or signals may decrease. In particular, when mono- or disaccharides are included, it is highly recommended that glycoproteins should be purified prior to the analysis by desalting and/or solvent replacement. The following is an example of a protein solution containing sucrose. Analytical conditions are described in the next page.

1) Fetuin sample containing 1% sucrose

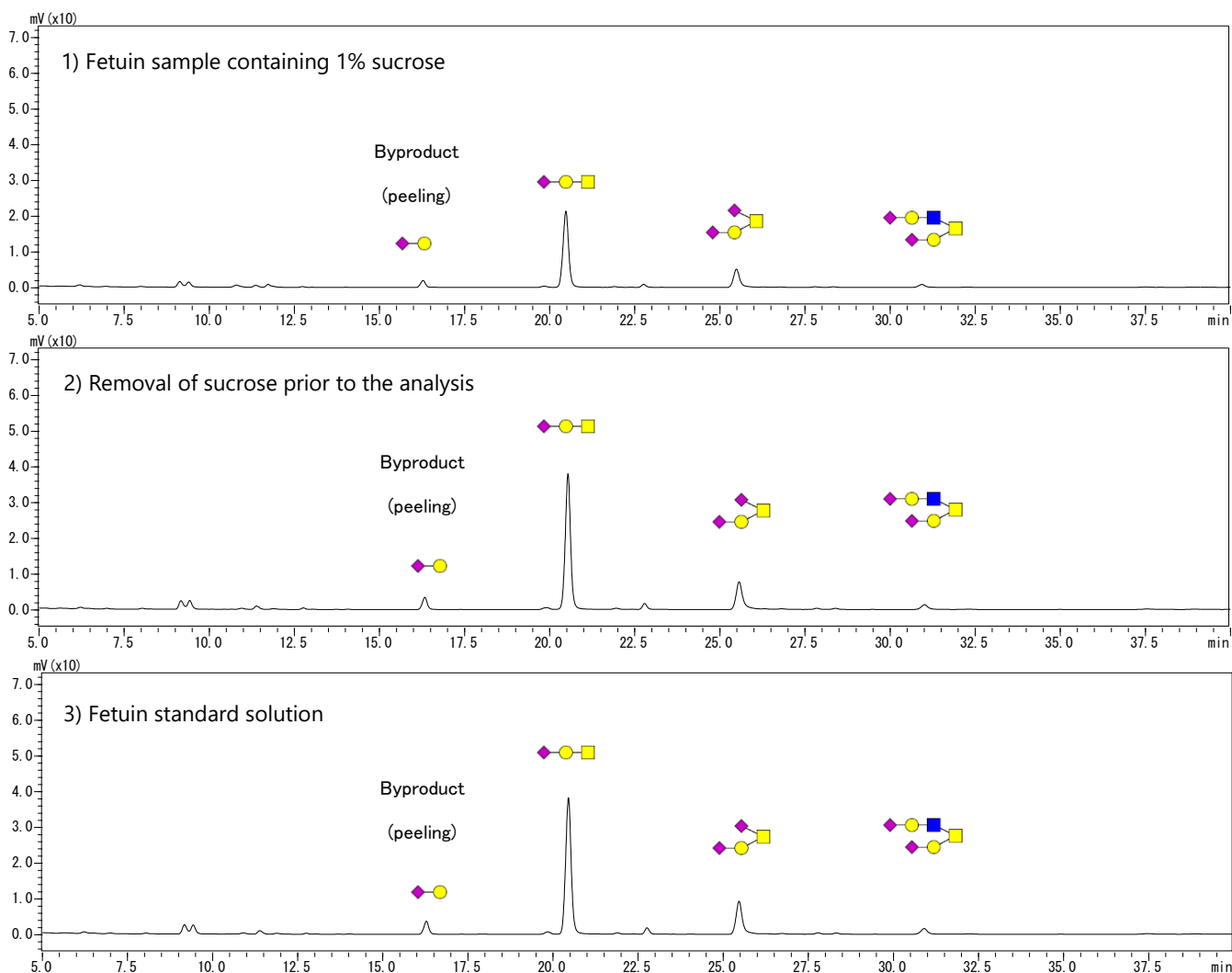
Solution containing 1% sucrose was treated with the kit, and HPLC analysis was performed using 1 μ L of 50 μ L of the recovered solution. The signals from O-glycans in fetuin decreased due to poor recovery and/or interference of sucrose.

2) Removal of sucrose prior to the analysis

Pretreatment of fetuin solution containing 1 % sucrose using a Zeba™ Micro Spin Desalting Column (Cat. No. 89877) manufactured by Thermo Scientific. Then this sample was treated with this kit, and HPLC analysis was performed using 1 μ L of 50 μ L of the recovered solution. The Signal derived from glycan can be recovered by treating this sample with this kit.

3) Fetuin standard solution

20 μ g Fetuin solution was treated with the kit, and HPLC analysis was performed using 1 μ L of 50 μ L of the recovered solution. (Analytical conditions are described in the next page.)



Material

Fetuin solution: Fetuin (Sigma, Cat. No. F3004) was adjusted to 2 mg/mL with 50 mM Ammonium Bicarbonate, and 10 μ L was treated.

Fetuin solution containing sucrose: Fetuin (Sigma, Cat. No. F3004) was adjusted to 2 mg / mL with 1% sucrose, 50 mM Ammonium Bicarbonate, and 10 μ L was treated.

Analysis conditions

LC system: Nexera, Shimadzu
Column: ACQUITY UPLC[®] Glycan BEH Amide, 1.7 μ m (2.1 x 150 mm)
Column temp.: 40 $^{\circ}$ C
Flow rate: 0.2 ml/min
Injection volume: 1 μ L
Fluorescence detection: Ex 330 nm/Em 420 nm using an RF-20Axs
Mobile phase A: 40% acetonitrile aq. containing 0.1% formic acid
Mobile phase B: 90% acetonitrile aq. containing 0.1% formic acid

Time (min)	%A	%B
0.00	0	100
50.00	100	0
65.00	100	0
65.01	0	100
80.00	0	100

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