Characterization of Released N-Glycans Using a Labeling Reagent for HILIC/Fluorecence -MS Analysis

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Streamlied Protocol (SOP)

Step I: Assay of Glycoproteins Preparation

	Preparation of Reagents：	Glycoprotein: 1.5 μg/μL Desalting mAb * 50 μL
A1	3% BT Surfatant w/ 5 mM TCEP solution： Pipette 150 μL 3% BT Surfactant into 0.5 mL size Centrifuge tube. Add 8 μL 100 mM TCEP. Mix well.	
A2	3% BT Surfatant solution: Dissolve 5 mg BT Surfactant in 150 μL 150 mM HEPES Buffer（store at -80 °C for up to 30 days）	
A3	150 mM HEPES Buffer (pH 7.9): Dissolve 714.9 mg HEPES in 20 mL dd-H2O. Adjust to pH 7.90 with 0.5 M NaOH.	
A4	100 mM TCEP： Dissolve 28.66 mg TCEP in 1mL dd-H_2O	
A-5	1.6 U/μL rPNGase F glycodase: Pipette 8 μL 10 units/μL rPNGase F into 42 μL water and mix well.	
A-6	N-Glycan Label Reagent: Dissolve 4.5 mg N-Glycan Label reagent in 55 μL anhydrous DMP (no more than 4 hours at 25 °C)	Tips: N-Glycan Label Reagent store at -80 °C and open the cap after warming to room temperature before use!

Item	Operating Description	Add Volume	Reagents	Facilities	Time	TEMP.	Remark
A-1	Pipette 10 μL 1.5 ug/μL mAb Desalting Glycoprotein into 0.6ml centrifuge tube.	10 μL	1.5 μg/uL desalting glycoprotein	0.5 mL centrifuge tube			
A-2	Add 10 μL 3% BT Surfactant w/ 5 mM TCEP Buffer (50mM HEPES, PH=7.9) mixed.	10 μL	3% BT Surfactant w/ 5 mM TCEP				
A-3	Heat to at least 95 °C for 3 min; then centrifuge for 1min and cool to room temperature			Heater	3 min	95 °C	
A-4	Add 10 μL 1.5 units/μL rPNGase F solution. Mix well and incubate for 15-30 min at 50 °C.	10 μL	1.5 units/μL rPNGase F solution	Heater	15-30 min	50 °C	
A-5	Cool to room temperature and leave for 5 min.				5min	25 °C	
A-6	Add 10 μL 82 μg/μL glycan labeling solution. Mix well and let it react for 5 min at room temperature	10 μL	82 μg/μL N-glycan Labeling solution	Vibrator	5 min	25 °C	
A-7	Add 15 μL anhydrous DMF and mix well.	15 μL	Anhydrous DMF	Vibrator			
A-8	Add 45 μL Acetonitrile and mix well.	45 μL	Acetonitrile	Vibrater	1 min		
A-9	Analyze labeled N-glycan samples with LC-FLR or LC-MS						

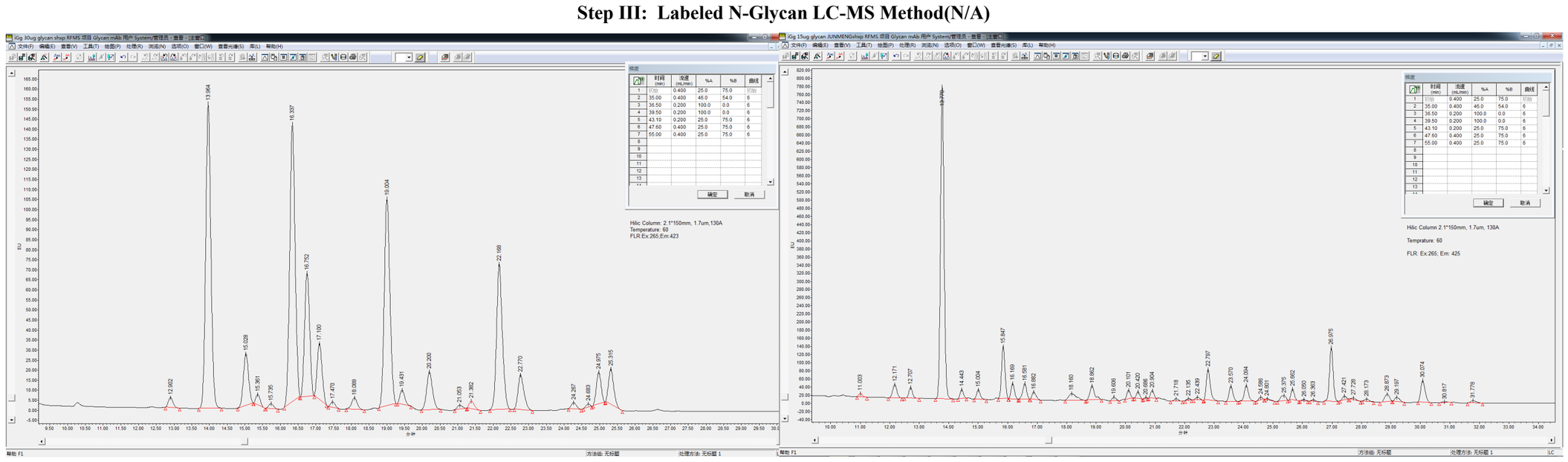
Tips: 1. Antibody samples must be desalted to prevent the interference of ionic affinity reagents with labeling process.

Step II: Method of HILIC-Fluorecence-MS for Labeled N-Glycan

	Mobile Phase：	Labeled Sample： 100ul
B1	Mobile Phase A： 50 mM Ammonium Formate solution（Dissolve 630 mg ammonium formate solid in 200 mL dd-water and add 160 μL MS Grade Formic acid (pH 4.4))	
B-2	Mobile Phase B： 100% Acetonitrile（recommend use LC-MS Grade ACN)	

	HPLC Column	Temperature	Fluorecence	Loading Volume	Flow Rate	Insert Tube	Needle wash
B-3							
B-4	Amide HILIC column, 130 Å, 1.7 μm, 2.1 x 150 mm	60 °C	EX 265/EM 425 nm	10 μL	0.40	100 μL	30% Water / 70% CAN
B-5							
B-6	LC Gradient Parameter	Time (min)	Flow Rate (mL/min)	%A	%B	Curve	
		0.00	0.40	25	75	6	
		35.0	0.40	46	54	6	
		36.5	0.20	100	0	6	
		39.5	0.20	100	0	6	
		43.1	0.20	25	75	6	
		47.6	0.40	25	75	6	
		55.0	0.40	25	75	6	

Step III: Labeled N-Glycan LC-MS Method(N/A)



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