

N-Glycan Kit 2.0 (single column)

Cat.No.: HYI146

Product Description

This document provides information regarding the general care and use of N-Glycan Kit for the fast enzymatic release and rapid labeling of N-glycans. This protocol is validated using monoclonal antibodies and has also been tested to perform for a wide range of other N-linked glycoproteins. We recommend the user to confirm enzymatic release for their particular sample.

Components

Module	Reagent	24 T
Deglycosylation Module	IgG	1.2 mg
	PNGase F	45 µL
	PNGase F buffer	0.5 mL
Labeling Module	MS reagent powder	8.2 mg×3
	Anhydrous DMF	1 mL
Clean-up Module	SPE column	8 pcs/pk×3
	Elution Buffer	5 mL
	Sample Diluent	6 mL
Collection Module	8-tube Strips (200µL)	100 pieces
	8-cap Strips	100 pieces
	Waste tray	1 pieces

Transportation and storage

Reagent	Storage
IgG	2-8°C, 12 months
PNGase F	-25~-15°C, 12 months , avoid freeze/thaw cycles
PNGase F buffer	2-8°C, 12 months
MS reagent Powder	2-8°C, 12 months, keep in dark
Anhydrous DMF	Room temperature, 12 months
Elution Buffer	Room temperature, 12 months

Sample diluent	Room temperature, 12 months
SPE column	Room temperature, once opened, the unused column need to be sealed.
200μL strips of 8 tubes, strips of 8 caps, waste tray	Room temperature

Instructions

User need to prepare below reagents and devices before you begin (One IgG sample (I4506) as example):

- 1) 0.85% NaCl, 100μL; acetonitrile (LC-MS grade); ultrapure water;
- 2) 15/85=ultrapure water/acetonitrile solution (v/v), 2mL; 1/9/90= formic acid/ultrapure water/acetonitrile (v/v), 20mL; 50 mM ammonium formate, PH=4.4, 500mL;
- 3) Heating module or metal bath (90°C and 50°C); vortex mixer.

1. Rapid Deglycosylation

- 1) Dilute IgG sample with 0.85% NaCl or UP water to get IgG final concentration at 2mg/mL.(the recommended amount can be adjusted according to the sample, testing equipment)
- 2) Add 20μL IgG solution to 200μL reaction tube, add 3μL PNGase F buffer, pipette up and down to mix and then add 3.3μL ultrapure water, aspirate and dispense to mix.
- 3) Add all above solution to heating module at 90°C, 3 minutes, denature it, and then get it out from the heating module, and cool for 3 minutes at room temperature.
- 4) Add 1.2μL PNGase F, aspirate and dispense to mix, heat 50°C, 5 minutes with heating module. User can prepare labeling reagent while waiting.
- 5) Get it out from the heating module, and then cool for 3 minutes at room temperature.

2. Rapid Labeling of Glycosylamines

- 1) Labeling reagent solution: dilute one vial labeling reagent (8.2mg) with anhydrous DMF (60.13μL), this solution need to be prepared before use, aspirate and dispense 5-10 times to ensure the reagent is dissolved fully.
- 2) Add 6μL labeling reagent solution to reaction tube, aspirate and dispense 5-10 times to ensure mixing, and then allow the labeling reaction to proceed at room temperature for 5 minutes.
- 3) Add 179 μL acetonitrile, mix for next step.

3. Clean-up of Labeled Glycosylamines

- 1) Set up SPE Manifold

- 2) Condition wells: add 200μL ultrapure water to the elution plate to condition the wells, collect the waste by the waste tray.
- 3) Equilibrate wells: add 200μL 15/85 water/acetonitrile solution (v/v) to equilibrate wells, collect the waste by waste tray.
- 4) Load sample: Load the acetonitrile diluted samples and collect the waste by waste tray.
- 5) Wash the wells with 600μL 1/9/90 formic acid/ultrapure water/acetonitrile (v/v) twice and collect the waste by waste tray.
- 6) Remove the waste tray, and change it to collection plate.
- 7) Wash the cell with 50μL elution buffer, elute glycans to the collection plate, and repeat this step.
- 8) Add 200μL(the recommended amount can be adjusted according to the sample, testing equipment) sample diluent, mix it

4. HILIC-FLR

- 1) Chromatography column: ACQUITY UPLC® Glycan BEH Amide, 130 Å, 1.7 μm, 2.1 x 150 mm (waters part # 186004742).
- 2) Temperature of the column: 60 °C
- 3) Liquid phase A: 50 mM ammonium formate solution (LC-MS grade is recommended), pH=4.4.
- 4) Liquid phase B: 100% acetonitrile (LC-MS grade is recommended).
- 5) Flow rate: 0.4 mL/min;
- 6) Gradient:

Time (min)	Flow rate (mL/min)	%A	%B	Curve
0	0.4	25	75	6
35	0.4	46	54	6
36.5	0.2	100	0	6
39.5	0.2	100	0	6
43.1	0.2	25	75	6
47.6	0.4	25	75	6
55	0.4	25	75	6

- 7) FLR wavelengths: EX 265/EM 425 nm
- 8) FLR sampling rate: 2 Hz
- 9) Injection vol.: 10μL (the recommended amount can be adjusted according to the sample, testing equipment)

Please note that above parameters based on the machine equipped with “ACQUITY® RDa” LCMS, the user can adjust the parameter accordingly on other equipment.

Notes

1. If the intermediate product system contains citrate, acetate, phosphate, Tris, SDS; or if the system's pH is lower than 4 or higher than 9, and slight precipitation occurs during the use of this kit, affecting the results, it is recommended to perform solution replacement on the sample system.

● **Replacement operation process:** (The following suggestions are based on the experimental equipment: DLAB, model: D1624R. Due to different equipment, consumables, and samples, adjustments can be made according to actual conditions, such as time and speed, etc.)

- 1) Prepare a 10kD ultrafiltration centrifuge tube, add 150μL of ultrapure water, and centrifuge at ≥ 13000 rpm for 5 minutes. (If a large residual volume is found in the ultrafiltration tube, it is abnormal and a new ultrafiltration tube should be replaced and repeat the operation)
- 2) Take 300μL of the sample solution into the 10kD filtration tube and centrifuge at 12000rpm for 3 minutes. Discard the lower layer liquid and add 300μL of ultrapure water to the upper layer retained solution, then centrifuge at 12000rpm for 3 minutes. Repeat this twice, and transfer all the upper layer retained solution to the centrifuge tube (or to transfer the liquid completely, the inner tube can be inverted into a new outer tube and centrifuged at 12000rpm for 3 minutes). (The protein concentration can be tested and adjusted to an appropriate concentration for N-glycan testing.)

● **Or increase the speed and follow the operation as follows:**

- 1) Prepare a 10kD ultrafiltration centrifuge tube, add 150μL of ultrapure water, and centrifuge at ≥ 13000 rpm for 5 minutes. (If a large residual volume is found in the ultrafiltration tube, it is abnormal and a new ultrafiltration tube should be replaced and the operation repeated.)
 - 2) Take 300μL of the sample solution into the 10kD filtration tube and centrifuge at 14000rpm for 2 minutes. Discard the lower layer liquid and add 400μL of ultrapure water to the upper layer retained solution, then centrifuge at 14000rpm for 2 minutes. Repeat this once, and transfer all the upper layer retained solution to the centrifuge tube (or to transfer the liquid completely, the inner tube can be inverted into a new outer tube and centrifuged at 14000rpm for 2 minutes). (The protein concentration can be tested and adjusted to an appropriate concentration for N-glycan testing.)
2. For your safety and health, please wear lab coat and gloves.
 3. Use clean tips each time.
 4. Please follow the manual to store and use the reagents.

5. The Clean-up module contains acetonitrile, which is flammable and toxic. When using it, strict safety regulations must be followed. It should only be used by trained and capable laboratory personnel who can handle such substances. The safe use of the product is entirely the responsibility of the purchaser and user. It is recommended that: (1) Ventilation: Operations should be carried out in a fume hood or a well-ventilated area to prevent vapor accumulation; (2) Personal protection: Wear a gas mask, chemical protective goggles, etc.; (3) Keep away from open flames, heat sources, and oxidants (such as perchlorates). Store at a temperature of no more than 30° C and a relative humidity of no more than 80%. Store separately from acids, bases, and flammable substances; (4) Emergency response for leakage: For small leaks, use activated carbon to absorb or rinse with a large amount of water. Wastewater should be professionally treated; (5) Avoid skin contact. If nausea, vomiting, chest tightness, or convulsions occur, seek medical attention immediately.

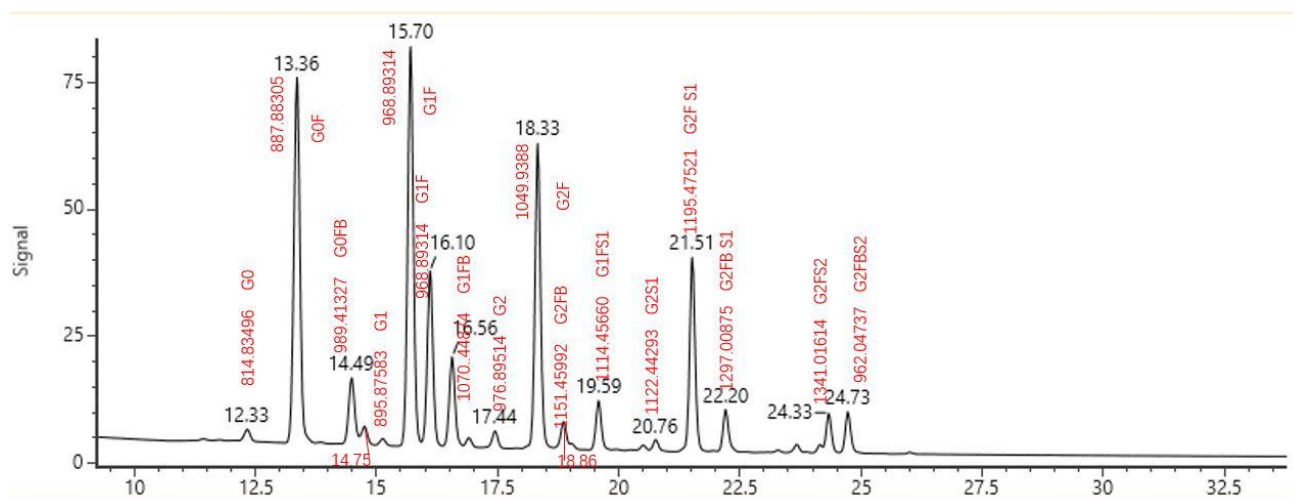


Figure: IgG data get from ACQUITY® RDa HILIC-FLR-MS