N-Glycan Kit (InstantPC)

Cat.No.: HYI144

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 and storage

Module	Reagent	24 T	96 T
Deglycosylation Module	IgG	1.2 mg	5 mg
	PNGase F	45 μL	150 μL
	PNGase F buffer	0.5mL	1 mL
	Surfactant (SF)	1.2mg*6	3.6 mg*8
Labeling Module	HIP powder	8.2 mg*3	24.6 mg*4
	Anhydrous DMF	1 mL	1 mL
Clean-up Module	Elution Plate (96 well)	1 piece	1 piece
	Elution Buffer	5 mL*1	5 mL*4
	Sample Diluent	5 mL*1	5 mL*4
Sample Collection Module	8-tube Strips (200μL)	100 pieces	100 pieces
	8-cap Strips	100 pieces	100 pieces
	Collection plate (96 well)	1 pieces	1 pieces
	Waste tray	1 pieces	1 pieces

Transportation and storage

Reagent	Storage
IgG	2-8℃, 12 months
PNGase F	-25~-15℃℃ , 12 months , avoid freeze/thaw cycles
PNGase F buffer	2-8℃, 12 months
Surfactant	Room Temperature, 12 months

HIP powder	2-8℃, 12 months, keep in dark
Anhydrous DMF	Room temperature, 12 months
Elution Buffer	Room temperature, 12 months
Sample diluent	Room temperature, 12 months
Elution Plate (96 well)	Room temperature， once opened, the unused well need to be sealed with the plastic paraffin film and keep desiccant at room temperature
200μL strips of 8tubes, strips of 8 caps, 96 well collection plate, waste tray	Room temperature，96 well collection plate be sealed and keep desiccant at room temperature

Instructions

Before starting this protocol, users should prepare the following reagents and equipment. The quantities listed below are based on processing one IgG sample.

1. 0.85% sodium chloride solution, 100 μL; Acetonitrile, LC-MS grade; Ultrapure water.
2. Ultrapure water/acetonitrile (15/85, v/v), 2 mL; Formic acid/ultrapure water/acetonitrile (1/9/90, v/v/v), 20 mL; 50 mM ammonium formate, pH 4.4, 500 mL.
3. SPE vacuum pump; Vacuum or positive-pressure manifold for 96-well plates; Heating block or dry bath capable of maintaining 90°C and 50°C; Vortex mixer; Micropipettes.

1. Rapid Deglycosylation

1) Weigh the IgG sample powder and dissolve it in 0.85% sodium chloride solution to obtain a final IgG concentration of 2 mg/mL.
2) Prepare the glycan release buffer: Dissolve one vial of surfactant (1.2 mg/3.6 mg) in 24 μL/72 μL of PNGase F buffer. Mix thoroughly and keep at room temperature. Prepare this buffer fresh before use.
3) Transfer 20 μL of the IgG solution into a 200 μL reaction tube. Add 3 μL of glycan release buffer and mix thoroughly by pipetting. Then add 3.3 μL of ultrapure water and mix thoroughly by repeated pipetting.
4) Incubate the mixture in a heating block at 90°C for 3 min for denaturation. Remove the tube and allow it to cool at room temperature for 3 min.
5) Add 1.2 μL of PNGase F and mix thoroughly by repeated pipetting. Incubate the mixture in a heating block at 50°C for 5 min. The labeling reagent may be prepared during this incubation period.
6) After the reaction is complete, remove the tube and allow it to cool at room temperature for 3 min.

2. Rapid Labeling of Glycosylation

1) Prepare the labeling reagent: Dissolve one vial of labeling reagent (8.2 mg/24.6 mg) in 60.13 µL/180.4 µL of anhydrous DMF. Prepare the labeling reagent fresh before use. Pipette up and down 5–10 times until completely dissolved.
2) Add 6 µL of the labeling reagent solution to the reaction tube. Pipette up and down 5 times to ensure thorough mixing, and incubate at room temperature for 5 min.
3) Add 179 µL of acetonitrile and mix thoroughly. The sample is then ready for purification.

3. Clean-up of Labeled Glycosylamines

1) Set up the vacuum or positive-pressure manifold and install the 96-well extraction plate.
2) Activation: Add 200 µL of ultrapure water to each well to be used for activation. Collect the flow-through in the waste tray.
3) Equilibration: Add 200 µL of water/acetonitrile (15/85, v/v) to each well for equilibration. Collect the flow-through in the waste tray.
4) Sample Loading: Load the samples diluted with acetonitrile onto the corresponding wells. Collect the flow-through in the waste tray.
5) Washing: Wash each well twice with 600 µL of formic acid/ultrapure water/acetonitrile (1/9/90, v/v/v). Collect the wash solution in the waste tray.
6) Remove the waste tray and replace it with a collection plate.
7) Elution: Add 50 µL of elution buffer to each well to elute the glycans into the collection plate. Repeat the elution twice.
8) Add 200 µL of sample diluent (recommended volume; adjust as appropriate according to the sample type, analytical instrument, and other experimental conditions). Mix thoroughly. The samples are then ready for instrumental analysis.

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：

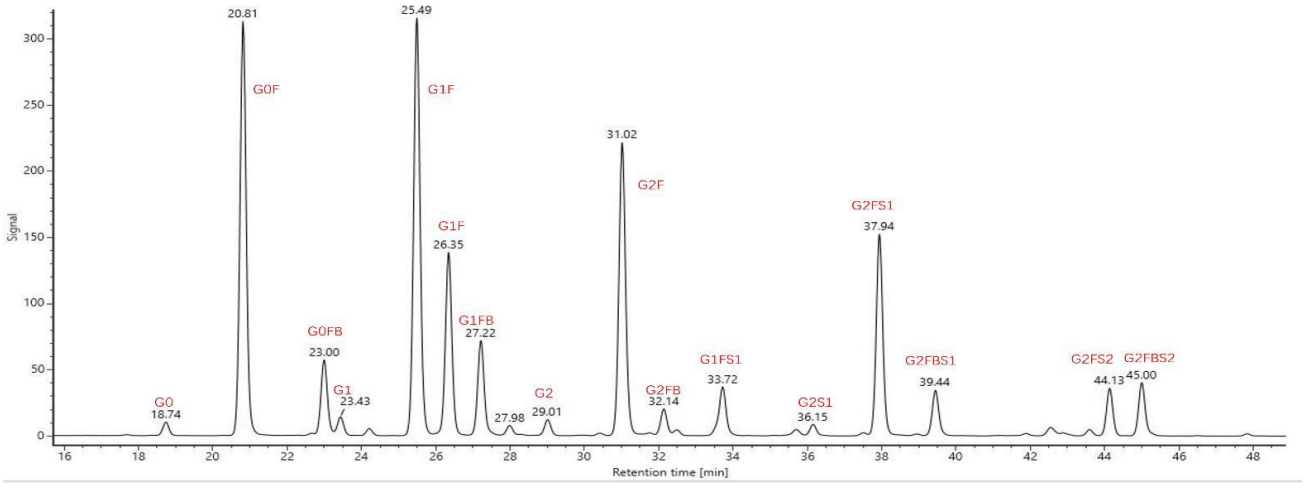
Time（min）	Flow rate (mL/min)	%A	%B	Curve
0	0.4	20	80	6
2	0.4	20	80	6
2.5	0.4	25	75	6
50	0.4	38	62	6
52	0.4	60	40	6
53	0.4	60	40	6
55	0.4	20	80	6
60	0.4	20	80	6

1) FLR wavelengths: EX 285 / EM 345 nm
2) FLR sampling rate: 2 Hz
3) Injection vol.: 10μL (the recommended amount can be adjusted according to the sample, testing equipment)

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

Notes

1. For your safety and health, please wear lab coat and gloves.
2. Use clean tips each time.
3. Please follow the manual to store and use the reagents.



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