HYASEN BIOTECH
华 晨 生 物

N-Glycan Kit (2AB-magnetic beads)

Cat.No.: HYI143

Product Description

This protocol provides instruction and information for pretreatment methods of N-glycan analysis from antibody drugs or glycoproteins. We develop a novel purification procedure that magnetic bead extraction combined with 2-AB(2-aminobenzamide) fluorescent labeling for enzymatically released glycans. This protocol was validated using monoclonal antibodies, and we recommend users to optimize it according to their own needs.

Components and storage

Module	Reagent	50 T	100 T	Storage
Deglycosylation Module	Co-enzyme	180μL	360μL	2-8℃
	PNGase F buffer	240μL	480μL	-25～-15℃
	PNGase F	120μL	240μL	-25～-15℃
Labeling Module	2-AB solution	0.5mL	1mL	-25～-15℃
	Reductant	0.5mL	1mL	-25～-15℃
Clean-up Module	Magnetic bead	12mL	24mL	2-8℃
	Washings	48mL	48mL*2	2-8℃
	Elution Buffer	1.2mL	2.4mL	2-8℃
	Sample Diluent	3.6mL	7.2mL	2-8℃
	Filter tube	50 pieces	100 pieces	2-8℃

Instructions

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

1. Acetonitrile (LC-MS grade)；
2. 20% formic acid (v/v); ultrapure water; 50 mM ammonium formate, PH=4.4, 500mL；
3. Magnetic Stand (200μL or 1.5mL); Heating module or metal bath (50℃); vortex mixer； pipette.

1. Rapid Deglycosylation

1) Dilute IgG sample with water to make final concentration at 2mg/mL(the concentration could be adjusted according to the real sample).

2) Pipette 200μL of magnetic bead suspension into a centrifuge tube(200μL or 1.5mL). And place on the magnetic stand for about 1 min, then removes storage solution carefully after the beads are pulled to the side of the vial, finally, removes the magnetic stand.
3) Add 20μL IgG solution to the reaction tube(slowly along the upper side of the magnetic bead), followed by adding 4μL PNGase F buffer and 2μL PNGase F, Then mix with the beads using a vortex and incubate at 50℃ for 10min.
4) Take it out from the heating module, and cool for 3 minutes to room temperature. Add 3μL Co-enzyme for 50℃ for 10min.

2. Rapid Labeling of Glycosylation

1) Labeling working solution: Mix the 2-AB solution, Reductant, and 20% formic acid as 1:1:1(v/v/v).
2) Pipette 25μL labeling working solution into the reaction tube, mix with the vortex and incubate at 65℃ for 15min.

3. Clean-up of Labeled Glycosylamines

1) Pipette 330μL acetonitrile into the reaction tube, mix with the vortex carefully and place on the magnetic stand, then removes supernatant carefully after the beads are pulled to the side of the vial, finally, removes the magnetic stand.
2) Pipette 400μL washings into the reaction tube, mix with the vortex carefully and place on the magnetic stand, then removes supernatant carefully after the beads are pulled to the side of the vial, finally, removes the magnetic stand. Repeat this step once.
3) Add 20μL(the volume could be adjusted according to the real sample) Elution Buffer into the reaction tube((slowly along the upper side of the magnetic bead)), mix with the vortex carefully and incubate for 3min at room temperature. Then place on the magnetic stand, removes supernatant carefully to another new tube after the beads are pulled to the side of the vial,
4) Add 60μL(the volume could be adjusted according to the real sample) sample Diluent into the new tube, mix with the vortex carefully and then load in 10μLor 1μL(the loading volume could be adjusted according to the real sample) to UPLC test. (users could choose to filter the mix sample using a filter tube before UPLC)

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

1) FLR wavelengths: EX 330/EM 420 nm

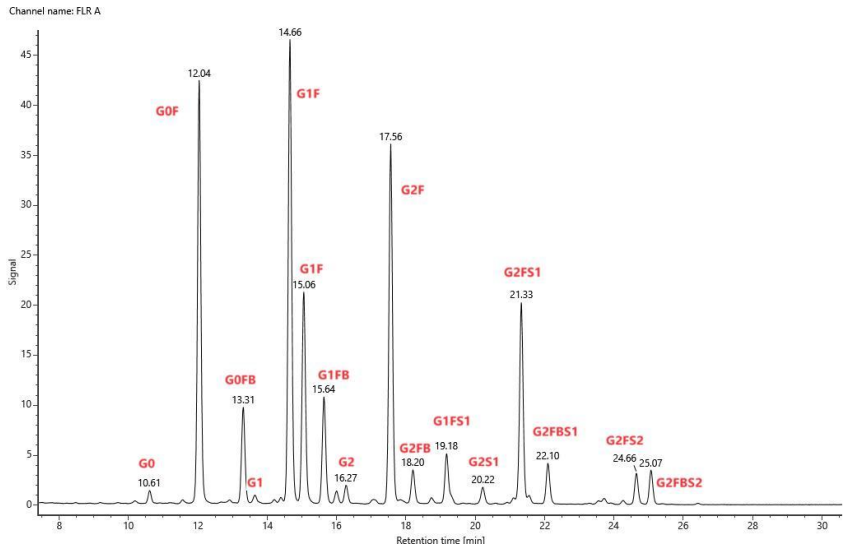
2) FLR sampling rate: 2 Hz

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 data by ACQUITY® RDa HILIC-FLR