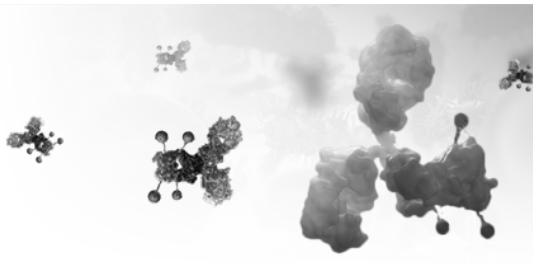

N-Glycan Analysis Kit (DCQA-2308-1)

This product is for research use only and is not intended for diagnostic use.

PRODUCT INFORMATION

Target	N-Glycan
Size	5 Samples/Kit
Storage	IgG , 2-8°C, 12 months PNGase F, -25~-15°C, 12 months, avoid repeated freezing and thawing; PNGase F buffer, 2-8°C, 12 months; Surfactant, room temperature, 12 months Marker, 2-8°C, 12 months DMF , room temperature, 12 months Wash buffer and sample diluent, room temperature, 12 months; Extraction column, room temperature.
Sample	Monoclonal antibodies
Intended Use	CD provides information on routine care and use of the N-Glycan Assay Kit for N-Glycan Rapid Enzyme Release and Rapid Labeling. This protocol was validated using monoclonal antibodies and has been tested for a variety of other N-linked glycoproteins. We recommend that users confirm the enzyme release for their specific samples.
Reagents And Materials Provided	1. IgG 2. PNGase F 3. PNGase F buffer 4. Surfactant 5. Marker 6. DMF 7. Wash buffer 8. Sample diluent 9. Extraction column
Materials Required But Not Supplied	Take 1 sample as an example (1) 0.85% NaCl 100μL; Acetonitrile (LCMS); Ultrapure water;

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(2) 15/85 water/acetonitrile solution 2mL; 1/9/90 formic acid/ultrapure water/acetonitrile 20mL; 0.2mL PCR tube, 1.5mL centrifuge tube; PCR machine.

Assay Procedure

Step 1: Rapid Glycosyl Release

(1) Weigh the IgG sample powder into 0.85% NaCl solution so that the final IgG concentration is 2 mg/mL.

(2) Glycosyl release buffer: Weigh 3 mg of surfactant and dissolve in 60 µL of PNGase F buffer, mix well and place at room temperature (this buffer is ready for use).

(3) Add 20 µL of IgG sample to a PCR tube, add 3 µL of glycosyl release buffer, mix by pipetting, then add 3.3 µL of ultrapure water, pipette and disperse to make it evenly mixed.

(4) Heat the above mixture on a PCR instrument at 90°C for 3 minutes to denature it, then take out the PCR tube and cool it at room temperature for 3 minutes.

(5) Add 1.2 µL PNGase F, aspirate and disperse to make it evenly mixed, and incubate the mixture on a PCR machine at 50°C for 5 min. During this waiting period, labeling reagents can be prepared.

(6) After the reaction is complete, take out the PCR tube and cool it at room temperature for 3 minutes.

Step 2: Rapid labeling of glycans

(1) Labeling reagent: Weigh 1.36mg maker into 10 µL anhydrous DMF (6uL is required for each sample, adjust the preparation amount according to the number of samples, the labeling reagent is prepared and used immediately).

(2) Add 6 µL of labeling reagent solution to the mixture in the PCR tube, aspirate and disperse 5 times to ensure mixing, and react at room temperature for 5 minutes.

(3) Add 179 µL of acetonitrile and mix well for purification.

Step Three: Purification

(1) Prepare solid phase extraction column, 1mL/5mL pipette gun, waste liquid cup, and clean 1.5mL centrifuge tube.

(2) Activation: Take 200µL of ultrapure water and add it to the small column, and use a pipette gun to slowly and uniformly pump the liquid from above to the waste liquid cup.

(3) Equilibration: Take 200µL of 15/85 water/acetonitrile solution to equilibrate the column (same method as above).

(4) Sample loading: Load the sample diluted with acetonitrile, with a total volume of about 200 µL.

(5) Take 600µL of 1/9/90 formic acid/ultrapure water/acetonitrile to wash the column twice to the waste cup.

(6) Take away the waste liquid cup and replace it with a clean 1.5mL centrifuge tube.

(7) Add two portions of 50µL elution buffer to the small column, pipette slowly and at a constant speed, and elute sequentially into clean centrifuge tubes.

(8) Add 200µL (recommended amount, which can be adjusted according to the sample, testing equipment, etc.) of sample diluent, mix well, and wait for the test on the machine.

Step 4: HILIC-FLR detection and analysis

(1) Column ACQUITY UPLC® Glycan BEH Amide, 130 Å, 1.7 µm, 2.1 × 150 mm (waters p/n 186004742).

(2) Column temperature: 60°C.

(3) Mobile phase A: 50 mM ammonium formate (LC-MS grade is recommended) solution,

	pH=4.4. (4) Mobile phase B: 100% acetonitrile (LC-MS grade is recommended). (5) Flow rate: 0.4 mL/min; (6) Gradient: (7) FLR wavelength: EX 265/EM 425 nm; (8) FLR sampling rate: 2 Hz; (9) Injection volume: 10μL (recommended volume, which can be adjusted according to samples, testing equipment, etc.). **Note: The above parameters are based on a mass spectrometer equipped with an "ACQUITY® RDa" detector.**
Typical Standard Curve	Example of Fluorescent Signal Intensity: Example of total ion current:
Precautions	1. Please wear a lab coat and gloves. 2. Use clean pipette tips each time you dispense. 3. Please store and use relevant reagents according to the instructions.

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