

KRISHZYME™ N-Glycan Kit

REF : KPGF-400-1

Ver 1.1

RIUO

RIUO	For Research & Industrial Use Only	REF	Catalog Number
	Store At	LOT	Batch Code
	Manufactured By		Biological Risk
	Expiry Date		Consult Operating Instructions

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Product Description:

The N-Glycan Kit is designed for the fast enzymatic release and rapid labeling of N-glycans. The protocol is validated using monoclonal antibodies and has also been tested with a wide range of other N-linked glycoproteins. Users should confirm enzymatic release for their particular sample.

Pack Size:

5 tests / Kit

Kit Contents:

Module	Component	Description
Deglycosylation Module	IgG	For troubleshooting and process validation
	PNGase F	For rapid deglycosylation
	PNGase F buffer	For rapid deglycosylation
	Surfactant	Enzyme-friendly surfactant used to denature glycoproteins
Labeling Module	MS reagent powder	For sample labeling
	Anhydrous DMF	As the resolution reagent of label
Clean-up Module	SPE column	For removing potential interferences
	Elution Buffer	For optimum glycan recovery
	Sample Diluent	For diluting SPE eluate

Shipping and Storage:

Reagent	Storage
IgG	2-8°C, 12 months
PNGase F	-25 to -15°C, 12 months; avoid freeze/thaw cycles
PNGase F buffer	2-8°C, 12 months
Surfactant	Room temperature, 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, seal unused columns

Protocol:

Before beginning, prepare the following reagents and devices. One IgG sample is used as the example:

- 0.85% NaCl, 100 µL; acetonitrile (LC-MS grade); ultrapure water.
- 15/85 ultrapure water/acetonitrile solution (v/v), 2 ml; 1/9/90 formic acid/ultrapure water/acetonitrile (v/v), 20 mL; 50 mM ammonium formate, pH 4.4, 500 mL.
- SPE vacuum pump, SPE manifold, heating module or metal bath (90°C and 50°C), vortex mixer, and pipette.

Step 1: Rapid Deglycosylation

- Dilute the IgG sample with 0.85% NaCl to obtain a final IgG concentration of 2 mg/ml.
- Prepare PNGase F buffer by diluting one vial of surfactant (3 mg) with 60 ul PNGase F buffer. Mix and store at room temperature. Prepare this solution before use.
- Add 20 ul IgG solution to a 200 ul reaction tube. Add 3 ul PNGase F buffer, pipette up and down to mix, then add 3.3 ul ultrapure water and mix.
- Place the tube in a heating module at 90°C for 3 minutes to denature the sample. Remove from the heating module and cool for 3 minutes at room temperature.
- Add 1.2 ul PNGase F, aspirate and dispense to mix, then heat at 50°C for 5 minutes. The labeling reagent can be prepared while waiting.
- Remove from the heating module and cool for 3 minutes at room temperature.

Step 2: Rapid Labeling of Glycosylamines

- Prepare labeling reagent solution by diluting one vial of labeling reagent (1.36 mg) with 10 ul anhydrous DMF immediately before use. Aspirate and dispense 5-10 times to ensure the reagent is fully dissolved.
- Add 6 ul labeling reagent solution to the reaction tube. Aspirate and dispense 5-10 times to mix, then allow the labeling reaction to proceed at room temperature for 5 minutes.
- Add 179 ul acetonitrile and mix for the clean-up step.

Step 3: Clean-up of Labeled Glycosylamines

- Set up the SPE manifold.
- Condition wells by adding 200 ul ultrapure water to the SPE column and collect waste in the waste tray.
- Equilibrate wells by adding 200 ul 15/85 water/acetonitrile solution (v/v) and collect waste in the waste tray.
- Load the acetonitrile-diluted sample and collect waste in the waste tray.
- Wash wells twice with 600 ul 1/9/90 formic acid/ultrapure water/acetonitrile (v/v), collecting waste in the waste tray.
- Remove the waste tray and replace it with a 1.5 ml centrifuge tube.
- Wash the cell with 50 ul elution buffer, elute glycans to the collection plate, and repeat this step.
- Add 200 ul sample diluent and mix. The recommended amount may be adjusted according to the sample and testing equipment.

Step 4: HILIC-FLR

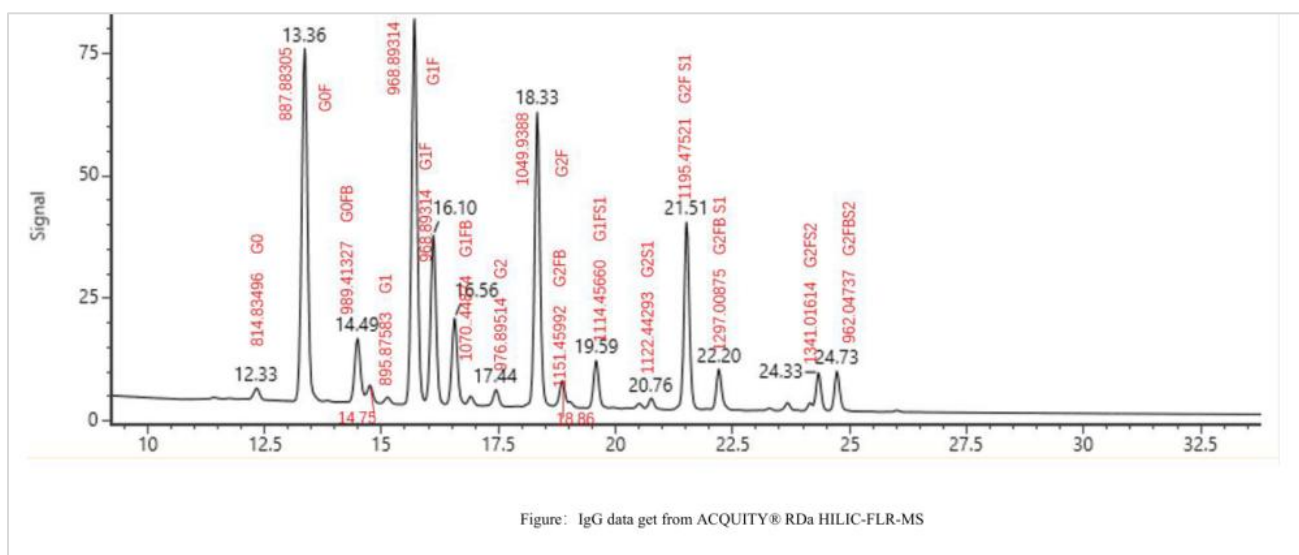
- Chromatography column: ACQUITY UPLC Glycan BEH Amide, 130 Å, 1.7 um, 2.1 x 150 mm (Waters part #186004742).
- Column temperature: 60°C.
- Liquid phase A: 50 mM ammonium formate solution, pH 4.4 (LC-MS grade recommended).
- Liquid phase B: 100% acetonitrile (LC-MS grade recommended).
- Flow rate: 0.4 mL/min.

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

- FLR wavelengths: EX 265/EM 425 nm.
- FLR sampling rate: 2 Hz.
- Injection volume: 10 ul.

Please note that the above parameters are based on equipment with ACQUITY RDa LC-MS. Users may adjust the parameters according to their equipment.



Notes:

- For safety and health, wear a lab coat and gloves.
- Use clean tips each time.
- Follow the manual for storage and use of the reagents.

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