

KRISHZYME™ KriGly® N-Glycan Workflow Kit (2-AB)

REF : KPGF-600-1 | KPGF-600-2

Ver 1.0

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 KRISHZYME™ KriGly N-Glycan Workflow Kit (2-AB) is designed for the rapid enzymatic release, fluorescent labeling and clean-up of N-glycans from antibody drugs and other glycoproteins. N-glycans are released by PNGase F in the presence of magnetic beads, labeled with 2-AB (2-aminobenzamide), and purified by magnetic-bead extraction, giving samples ready for HILIC-FLR or LC-MS analysis. The protocol is validated using monoclonal antibodies; users should optimize it according to their own samples.

Pack Size:

KPGF-600-1: 50 tests / Kit | KPGF-600-2: 100 tests / Kit

Kit Contents:

Module	Component	Description	KPGF-600-1 (50 T)	KPGF-600-2 (100 T)
Deglycosylation Module	PNGase F buffer	Reaction buffer for deglycosylation	240 µl	480 µl
Labeling Module	PNGase F	Enzyme for rapid release of N-glycans	120 µl	240 µl
	2-AB solution	2-aminobenzamide fluorescent label	0.5 ml	1 ml
	Reductant	Reducing agent for the labeling reaction	0.5 ml	1 ml
Clean-up Module	Magnetic beads	For on-bead deglycosylation and glycan clean-up	12 ml	24 ml
	Washings	Removes excess label and reaction reagents	48 ml	2 × 48 ml
	Elution Buffer	Elutes labeled glycans from the beads	1.8 ml	3.6 ml
	Sample Diluent	Dilutes the eluate for HILIC injection	3.6 ml	7.2 ml
	Filter tubes	Optional filtration before injection	50 pcs	100 pcs

Shipping and Storage:

Reagent	Storage
PNGase F buffer	-25 to -15°C
PNGase F	-25 to -15°C; avoid repeated freeze/thaw cycles
2-AB solution	-25 to -15°C
Reductant	-25 to -15°C
Magnetic beads	2-8°C
Washings	2-8°C
Elution Buffer	2-8°C
Sample Diluent	2-8°C
Filter tubes	2-8°C

Protocol:

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

- Acetonitrile (LC-MS grade); 20% formic acid (v/v); ultrapure water.
- 50 mM ammonium formate, pH 4.4, 500 ml.
- Magnetic stand (for 200 µl or 1.5 ml tubes); heating module or metal bath (50°C and 65°C); vortex mixer; pipettes.

Step 1: Rapid Deglycosylation

- Dilute the IgG sample with ultrapure water to a final concentration of 5 mg/ml. The concentration may be adjusted according to the sample.
- Pipette 200 µl of magnetic bead suspension into a 200 µl or 1.5 ml tube. Place on the magnetic stand for about 1 minute; once the beads have collected on the side of the tube, carefully remove and discard the storage solution. Remove the tube from the magnetic stand.
- Add 20 µl IgG solution slowly along the upper side of the bead pellet, then add 4 µl PNGase F buffer and 2 µl PNGase F.
- Vortex to mix with the beads and incubate at 50°C for 10 minutes.

Step 2: Rapid 2-AB Labeling of Released Glycans

- Prepare the labeling working solution by mixing 2-AB solution, Reductant and 20% formic acid at 1:1:1 (v/v/v).
- Add 25 µl labeling working solution to the reaction tube, vortex to mix and incubate at 65°C for 15 minutes.

Step 3: Clean-up of 2-AB Labeled Glycans

- Add 330 µl acetonitrile to the reaction tube and vortex carefully. Place on the magnetic stand and, once the beads have collected on the side of the tube, carefully remove and discard the supernatant. Remove from the magnetic stand.
- Add 400 µl Washings, vortex carefully and place on the magnetic stand. Once the beads have collected, carefully remove and discard the supernatant. Remove from the magnetic stand. Repeat this wash once.
- Add 30 µl Elution Buffer slowly along the upper side of the bead pellet, vortex carefully and incubate for 3 minutes at room temperature. Place on the magnetic stand and transfer the supernatant containing the labeled glycans to a new tube. The elution volume may be adjusted according to the sample.
- Add 50 µl Sample Diluent to the eluate and vortex to mix. The sample is ready for UPLC analysis; if required, filter it through a Filter tube before injection.

Step 4: HILIC-FLR

- Chromatography column: ACQUITY UPLC Glycan BEH Amide, 130 Å, 1.7 µm, 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 330/EM 420 nm.
- FLR sampling rate: 2 Hz.
- Injection volume: 10 µl (or 1 µl); adjust according to the sample and instrument.

Please note that the above parameters are based on an ACQUITY I-Class UPLC equipped with an ACQUITY RDa detector. Users may adjust the parameters according to their own equipment.

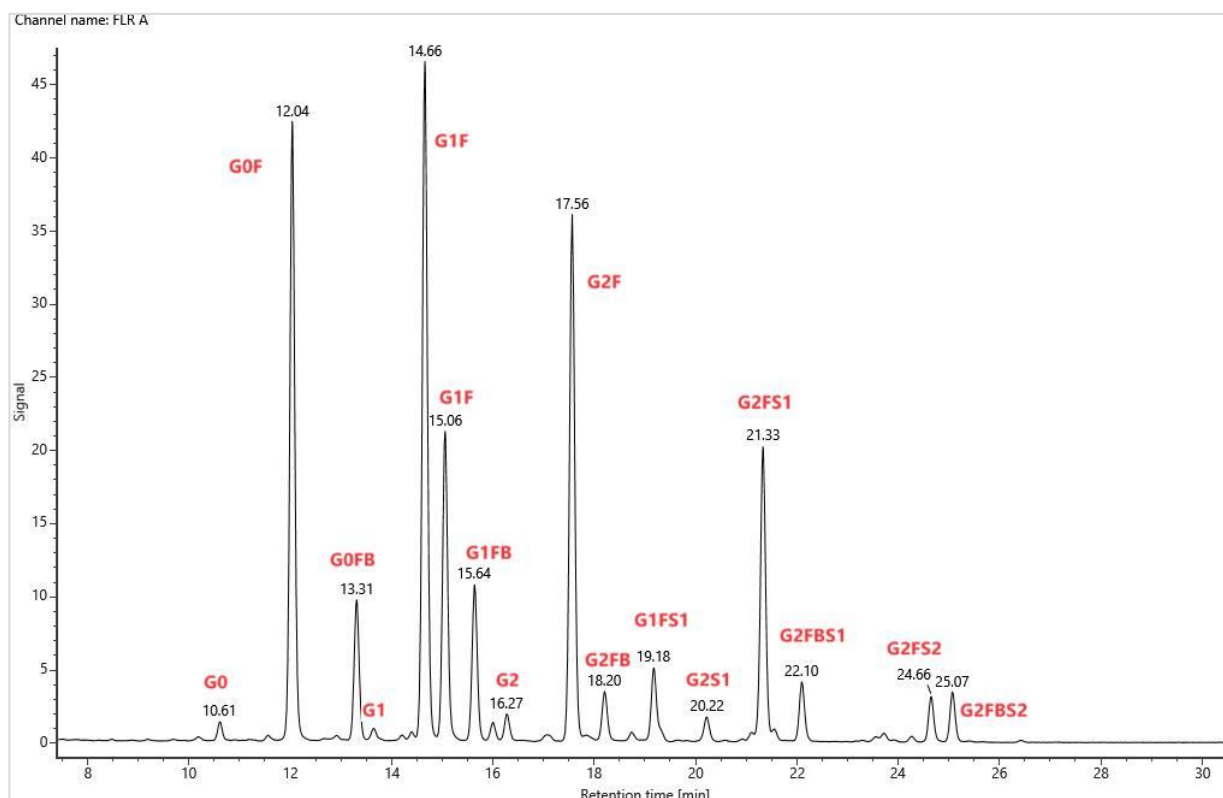


Figure: 2-AB labeled N-glycan profile of IgG by HILIC-FLR (ACQUITY RDa)

Notes:

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

- The clean-up procedure uses acetonitrile, which is flammable and toxic, and should be handled only by trained laboratory personnel. Work in a fume hood or well-ventilated area, wear chemical protective goggles and gloves, and keep away from open flames, heat sources and oxidizers (such as perchlorates).
- Store acetonitrile at no more than 30°C and 80% relative humidity, separately from acids, bases and flammable substances.
- For small spills, absorb with activated carbon or rinse with plenty of water. Waste should be handled by a professional waste-treatment service.
- Avoid skin contact. If nausea, vomiting, chest tightness or convulsions occur, seek medical attention immediately.
- This protocol was validated using monoclonal antibodies; users should optimize it for other glycoproteins.

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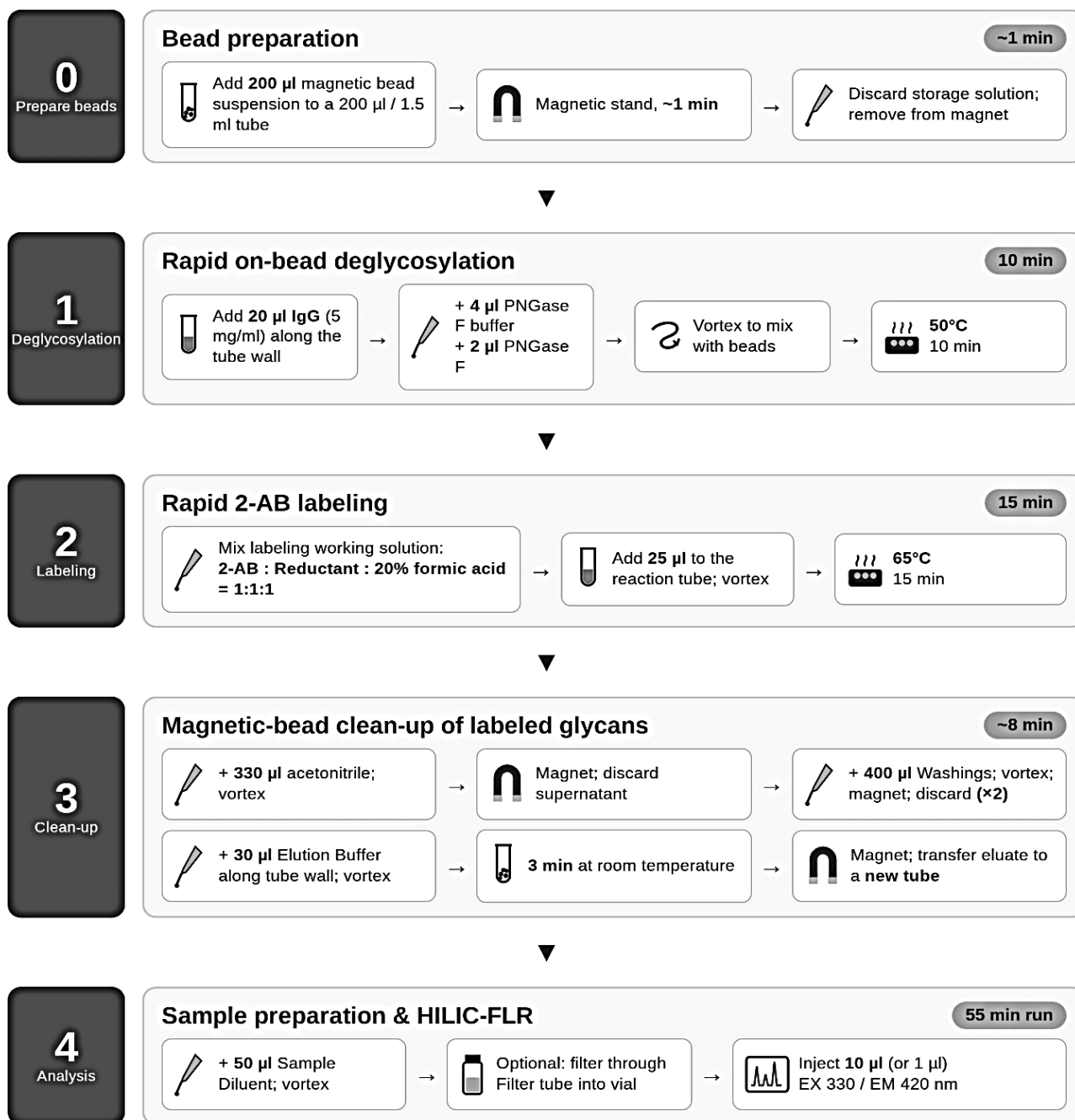
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KriGly N-Glycan Workflow Kit (2-AB)

Workflow schematic · KPGF-600-1 | KPGF-600-2 · IgG (5 mg/ml) as example sample

 Incubation time
~35 min
 sample to vial


Timeline

0 | 1 · Deglycosylation 50°C · 10 min | 2 · 2-AB labeling 65°C · 15 min | 3 · Clean-up ~8 min | 4 · Inject

HILIC-FLR conditions

 ACQUITY UPLC Glycan BEH Amide, 130 Å, 1.7 μ m, 2.1 × 150 mm · 60°C
 A: 50 mM ammonium formate, pH 4.4 · B: acetonitrile
 0.4 ml/min · 75 → 54% B over 35 min · 2 Hz

You will need

Acetonitrile (LC-MS grade) · 20% formic acid (v/v) · ultrapure water · 50 mM ammonium formate pH 4.4 · magnetic stand · heat block (50°C & 65°C) · vortex mixer · pipettes