

A Technical Comparison in N-Glycan Sample Preparation

KRISHZYME™ KriGly N-Glycan Workflow Kit (2-AB) vs. the traditional sold-separately 2-AB route from Competitor L* and Competitor Q*

OVERVIEW

What This Comparison Covers

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BACKGROUND

Two Routes to the Same 2-AB Profile

2-AB labelling with HILIC-FLR is the reference method for released N-glycan profiling of antibodies and other glycoproteins. Every route has to do the same four jobs before injection; the difference is how many products, vessels and hours it takes to do them.

- ✓ Release N-glycans with PNGase F
- ✓ Remove protein, salts and detergent
- ✓ Label each glycan's reducing end with 2-AB
- ✓ Remove excess dye before HILIC-FLR



Competitor Q distributes Competitor L*'s labelling kits, so the traditional route is one chemistry sold as four separate products. KriGly packages the same reductive-amination 2-AB chemistry as one kit, so results stay comparable with existing 2-AB methods and glycan libraries.*

WORKFLOW AT A GLANCE

7 Steps Across 3 Vessels vs. 4 Steps in One Tube

Traditional Route

4 products, 2 suppliers · 10–12 h denatured, 1.5–2 days native

Denature

SDS + β -ME at 100°C, 5 min; add Triton X-100

PNGase F release

37°C, 3 h · at least 24 h if native

EB10 post-release clean-up

separate cartridge: bind, wash, elute

Dry-down

centrifugal evaporator, kept at or below 28°C

2-AB labelling

open 3 ampoules, mix reagents · 65°C, 3 h

LudgerClean S clean-up

prime, spot, wash, elute · 80–100 min

Optional dry-down

evaporate 1 ml eluate, reconstitute

HILIC-FLR analysis: same 2-AB method for both routes · dashed boxes = dry-down steps KriGly does not need

KriGly KPGF-600

1 kit, 1 tube · about 35 min, native IgG

On-bead PNGase F release

native IgG, no denaturant · 50°C, 10 min

2-AB labelling

mix 3 ready solutions 1:1:1 · 65°C, 15 min

Magnetic-bead clean-up

acetonitrile, 2 washes, elute · about 8 min

Add Sample Diluent

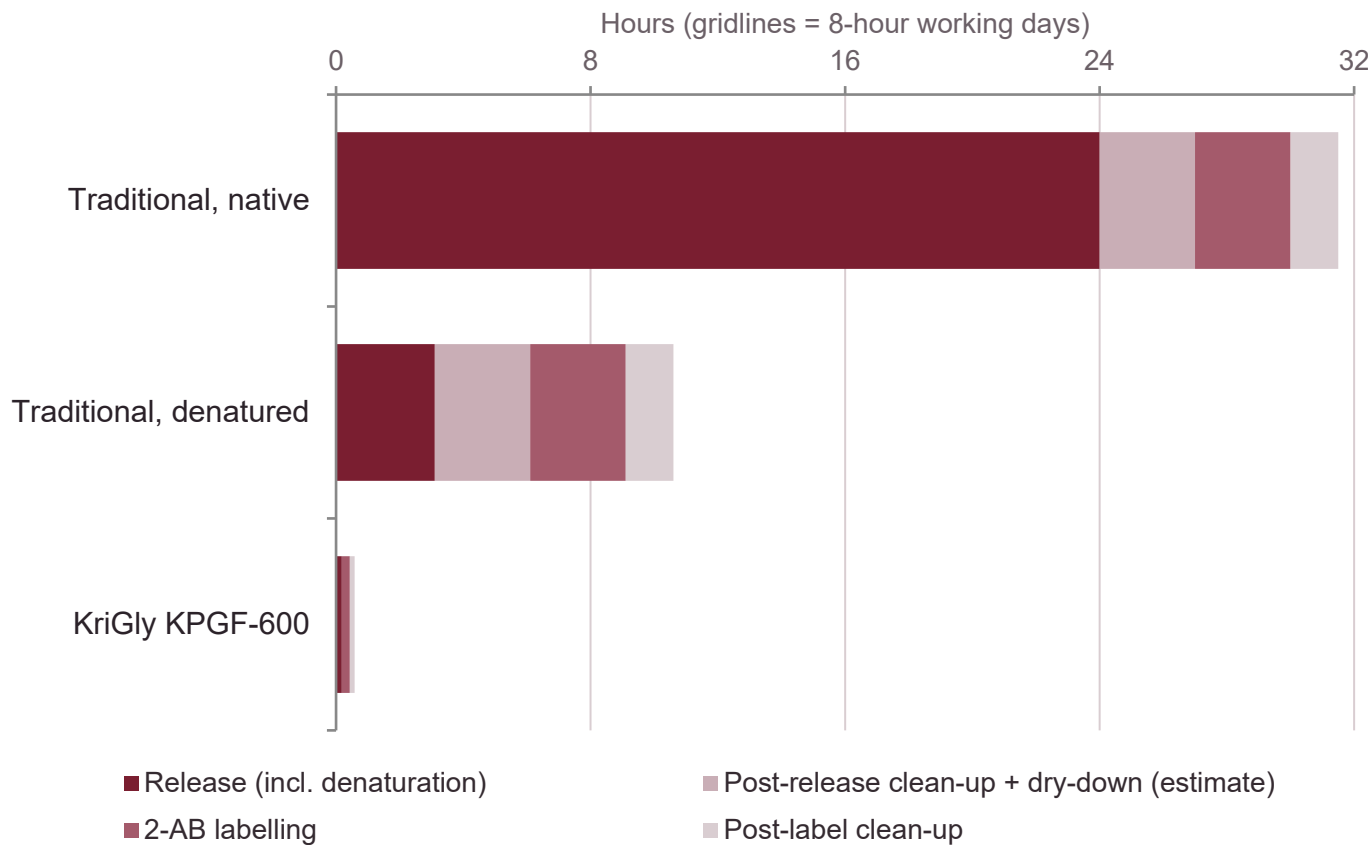
inject directly, no drying

Ready in about 35 min
no dry-downs, one transfer

TIME-FLOW

Injection-Ready in 35 Minutes, Not 10–31 Hours

Hours from glycoprotein to injection-ready 2-AB glycans. The traditional route is set by two long incubations; on native protein the overnight digest alone runs past a working day.



31.5 h

Traditional, native protein

24 h digest + clean-up + 3 h label

10.6 h

Traditional, denatured

3 h digest + clean-up + 3 h label

~35 min

KriGly KPGF-600

10 + 15 min incubations, ~8 min clean-up

Four Products from Two Catalogues vs. One Kit

Step	Traditional route (sold separately)	KriGly (KPGF-600)
N-glycan release	Competitor Q* PNGase F E-PNG01 + buffer, SDS/β-ME denaturant, Triton X-100; 100°C, then 37°C for 3 h (≥ 24 h native)	Deglycosylation module: PNGase F + buffer; native IgG, 50°C for 10 min on magnetic beads
Post-release clean-up	Competitor L* cleanup cartridges to strip protein, salts, detergent; glycans then dried down	No separate step: beads carry glycans into labelling
2-AB labelling	Competitor L* 2-AB labelling kit, DMSO, acetic acid, sodium cyanoborohydride in ampoules; 65°C for 3 h	Labelling module: 2-AB + reductant + 20% formic acid; 65°C for 15 min
Post-labelling clean-up	Competitor L* post labelling cleanup cartridges - prime, spot, adsorb, wash, elute; 80–100 min	Clean-up module: acetonitrile, two bead washes, elution; about 8 min
Ready for HILIC	Eluate in ~1 ml water, often dried and reconstituted	Eluate + Sample Diluent, injected directly
Products to order & qualify	4 products (1 to 2 suppliers)	1 product / workflow (1 supplier)

No Dry-Downs, No Cartridges, No Denaturant

Factor	Traditional Route	KriGly
Sample vessel	Tube → Cleanup cartridge → tube → Cleanup cartridge → tube	One tube until the final eluate
Dry-downs	Up to 2 (before labelling if > 10 µl; optional after S clean-up)	None
Denaturation	SDS + β-mercaptoethanol at 100°C, then Triton X-100	None: native protein
Labelling prep	Open 3 glass ampoules; mix DMSO + acetic acid, dissolve dye, dissolve reductant	Mix 3 ready solutions 1:1:1
Reductant	Sodium cyanoborohydride, toxic; fume cupboard advised	Kit reductant; acetonitrile handled in a fume hood
Equipment	Heat block (37, 65, 100°C), cartridge rack or vacuum manifold, centrifugal evaporator	Heat block (50, 65°C), magnetic stand, vortex
Where it goes wrong	Air gaps stop cartridge flow; sialic acid loss if drying runs warm (> 28°C) or acidic	Bead loss when aspirating supernatants

BY THE NUMBERS

What a Lab Stops Doing with KriGly

Per sample, from glycoprotein to injection-ready 2-AB glycans.



Vessels

3 → 1

tube, post deglycosylation
cleanup cartridge, post
labelling cleanup cartridge
vs. one tube



Dry-downs

2 → 0

no centrifugal evaporator
step



Solvent per sample

**~18 ml → ~1.2
ml**

Post Labelling cleanup
cartridge washes alone vs.
whole KriGly workflow



Products to order

4 → 1

two suppliers, four lots vs.
one kit

Source: Competitor L Post Labelling Cleanup guide (3 ml water, 3 ml ACN, 6 ml 96% ACN, 6 ml 30% acetic acid per cartridge); KriGly IFU volumes (330 µl ACN, 2 × 400 µl washes, 30 µl elution, 50 µl diluent).*

SENSITIVITY & DATA QUALITY

Same Label, Fewer Places to Lose Sample

Both routes put the same 2-AB label on the glycan, so per-mole fluorescence is the same. KriGly's advantage is how much glycan reaches the column, not a brighter signal. [We don't claim a brighter signal. We leave that to you to see the glycans and sensitivity.]

Factor	Traditional Route	KriGly
Label and detection	2-AB, fluorescence	2-AB, fluorescence (EX 330 / EM 420 nm)
Working range	25 pmol–25 nmol glycan per reaction (Ludger)	100 µg IgG per reaction (~1.3 nmol N-glycan)
Labelling efficiency	Typically > 85% (Competitor L*)	Not published by the supplier
Sialic acid integrity	< 2 mol% loss in labelling; drying is the main risk	No dry-down; 15-min acid labelling step
Loss points	2 cartridge cycles, up to 2 dry-downs, 3–4 transfers	1 bead cycle, 1 transfer
Demonstrated profile	Reference method for 2-AB HILIC	IgG profile, 16 annotated peaks, G0 to G2FBS2

Battlecard: At a Glance

	Traditional Route (Competitor L* / Q*)	KriGly KPGF-600
Time to injection-ready	10.6 h denatured · 31.5 h native	About 35 min, native
Products to order	4, from 2 suppliers	1 kit
Vessels / dry-downs	3 vessels / up to 2 dry-downs	1 tube / none
Denaturation	SDS + β -ME, 100°C	None
Solvent per sample	~18 ml (S clean-up alone)	~1.2 ml
Label	2-AB, reductive amination	2-AB, reductive amination
Consumables per sample	£87.19 ($\approx$ €102), Competitor L* List Price	€40.63–41.67 ($\approx$ £35–36), List Price

Let's Talk N-Glycan Workflows

Request a KriGly N-Glycan Workflow Kit evaluation, or a side-by-side run against your current 2-AB route.

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