

Product Guide for N-Glycan Release & Procainamide Labelling Workflow Module

(LT-KPROC-VP30-MOD)

The presented protocol is for the in-solution PNGase F release, procainamide labelling and clean-up of *N*-glycans from glycoproteins/glycopeptides. The protocol presents a golden standard methodology for the analysis of *N*-linked glycans using (U)HPLC, LC-MS or MALDI-MS approaches. Typical reaction conditions are demonstrated. For more detailed instructions and the troubleshooting guide, please refer to the following documentation:

- PNGase F release guide - <https://www.ludger.com/docs/products/lz/lz-rpngasef/ludger-lz-rpngasef-kit-guide.pdf>
- Procainamide labelling guide - <https://www.ludger.com/docs/products/lt/lt-kproc/ludger-lt-kproc-vp24-guide.pdf>
- S-cartridge clean-up - <https://www.ludger.com/docs/products/lc/s/ludger-lc-s-ax-guide.pdf>

Product	Product code	Quantity	Components
Main components			
PNGase F release kit	LZ-rPNGaseF-30	1	PNGase F enzyme
			Reaction buffer
			NP-40 surfactant
			Denaturant
Procainamide labeling kit with picoline borane	LT-KPROC-VP24	1	Procainamide dye
			Picoline borane reductant
			Acetic acid - DMSO
LudgerClean S cartridges	LC-S-A6	5	S cartridges
Process control			
Human IgG Glycoprotein Standard	GCP-IGG-100U	2	IgG glycoprotein standard
System suitability control			
Procainamide labelled glucose homopolymer ladder	CPROC-GHP-30	1	Procainamide GHP ladder

PNGase F release – LZ-rPNGaseF-kit

It is advisable to process the supplied human IgG glycoprotein (GCP-IGG-100U) as a positive control alongside the samples.

Use up to 100 µg of glycoprotein per replicate.

Sample denaturation

1. Dry samples down if the volume exceeds 9 µL. Make up sample volume to 9 µL with ultrapure water.
2. Add 1 µL of 10X Denaturation Solution, vortex and briefly centrifuge.
3. Incubate the samples at 100°C for 10 minutes, then cool to room temperature.

N-glycan release

4. Add 2 µl of 10X Reaction Buffer to each glycoprotein sample.
5. Add 2 µl of 10% NP-40 solution.

6. Adjust the reaction volume to 20 µl by adding 6 µl of water.
7. Add 1 µl of PNGase F, vortex and briefly centrifuge.
8. Incubate the samples at 37°C for 1-3h.

Procainamide labelling – LT-KPROC-VP24

Sample preparation

9. Dry down the samples. Make up sample volume to 10 µl with ultrapure water.

Preparation of labelling reagent

10. Add 150 µl of the 30% acetic acid in DMSO to a vial of dye and mix by pipette action until the dye is dissolved. Heat up to 65°C can be applied to aid dissolution.
11. Transfer the 150 µl of dye solution to a vial of reductant and mix by pipette action until the reductant is dissolved. Heat up to 65°C can be applied to aid dissolution.

Labelling

12. Add 10 µL of labelling reagent to each glycan sample, vortex and briefly centrifuge.
13. Incubate the samples at 65°C for 1h.

S-cartridge clean-up – LC-S-A6

Priming the S cartridges

14. Prime the S-cartridges by washing with 1 ml of water, followed by 5 ml of 30 % acetic acid and 1 ml of acetonitrile.

Samples clean-up

15. Spot the samples evenly onto the freshly washed, wet S-cartridges and allow to absorb for 15 minutes.
16. Rinse each sample vial with 100 µl acetonitrile and apply to the corresponding S-cartridge disc.
17. Wash the S-cartridges with 1 ml of acetonitrile, followed by 2 x 5 ml of acetonitrile.
18. Elute the glycans into a fresh vial with 2 x 0.5 ml water.

Sample analysis

Analyse the samples using a method of choice. If needed, concentrate the samples by drying them down, then dissolving in a desired volume of water or other solvent of choice. Use the supplied Glucose Homopolymer (CPROC-GHP-30) as a system suitability control or as an external calibrant.

Additional procainamide labelled *N*-glycan standards and glycan libraries (to be purchased separately) can be used as system suitability standards (to ensure correct separation and detection conditions) as well as reference standards (to be analysed alongside the samples for structural identification of

glycans). These can include complex di-, tri-, tetra-antennary (neutral as well as sialylated) and oligomannose glycan standards.