# Product # LT-KAB-VP30-MOD

Ludger Document # LT-KAB-VP30-MOD-Guide-v1.2

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# N-Glycan Release & 2-AB Velocity Labelling Module (LT-KAB-VP30-MOD)

The presented protocol is for the in-solution release, 2-AB 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
- 2-AB labelling guide - https://www.ludger.com/docs/products/lt/lt-kab/ludger-lt-kab-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 |
| 2-AB labelling kit with picoline borane | LT-KAB-VP24 | 1 | 2-AB 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** | | | |
| 2-AB labelled glucose homopolymer ladder | CAB-GHP-30 | 1 | 2-AB GHP ladder |

*LZ-rPNGaseF-30 is the 30 µL version of LZ-rPNGaseF-kit (150 µL).

## 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.

## 2-AB labelling – LT-KAB-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.

### Labeling

12. Add 10 µL of labeling 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 5 x 1 ml of 96% 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 (CAB-GHP-30) as a system suitability control or as an external calibrant.

Additional 2-AB 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.