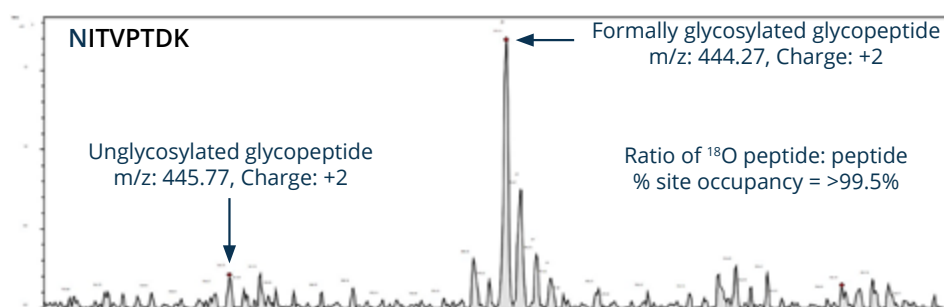


## Ludger Glycoprofiling Services: N-Glycan Site Occupancy Analysis

The amino acid sequence of the glycoprotein determines the number of potential N-glycosylation sites. As shown in the workflow below, the relative proportion of occupied N-glycan sites can be determined using a PNGase F enzyme in the presence of  $^{18}\text{O}$  water. Any peptide with an occupied N-glycan site will gain 3Da from the  $^{18}\text{O}$  incorporated by the enzyme. The relative occupancy proportion is obtained by analysing the digested sample using C18-LC-MS/MS or MALDI-TOF-MS/MS (see Figure 1).

Proteolysis	Glycan Release	Analysis	Report Contents
Reduction/alkylation and protease digestion	PNGase F + $^{18}\text{O}$ Water <b>LZ-rPNGaseF-kit</b> <b>E-PNG01</b> <b>E-PNG01-200</b> <b>E-rPNG01</b>	MALDI-TOF-MS/MS or C18-LC-MS/MS	1. C18-LC-MS/MS or MALDI-TOF-MS/MS spectra for the $^{18}\text{O}$ deglycosylated peptide sample
<b>Standards &amp; Controls</b> (run with your samples)			2. C18-LC-MS/MS or MALDI-TOF-MS/MS spectra for standards and controls
Human IgG glycoprotein standard <b>GCP-IGG-100U</b> KVANKT-A2G2S2 glycoprotein standard <b>BQ-GPEP-A2G2S2-10U</b>			3. Proportion of N-glycan occupancy at each glycosylation site 4. Summary of findings

Note that the analytical protocol must be adapted to each sample based on its amino acid sequence and buffer formulation.



**Figure 1.** LC-ESI-MS spectra showing the mass difference between occupied and unoccupied glycopeptide after PNGase F digestion in presence of  $^{18}\text{O}$  water.

### Used for:

- Quality control: batch-to-batch consistency
- Biosimilars: comparability studies

Our team has years of experience in glycoprofiling. They will choose the best parameters for your sample and run it alongside the appropriate **Ludger positive and negative controls, and system suitability standards**.

### Type of sample sources:

- Biopharmaceuticals such as mAbs and glycoprotein hormones (e.g. follicle stimulating hormone (FSH) and erythropoietin (EPO), Fc fusion proteins, vaccines)
- Cultured cells, clinical tissues, or fluids (e.g., SARS-CoV-2 infected cell lines and COVID-19 patient plasma and tissue samples).

Given its complexity, this type of analysis is offered at Ludger as a **Level 3 or advanced analytical module**.

If further information on the N-glycan composition at any specific glycosylation site is required, we recommend using Ludger's **Level 3 site-specific glycosylation analysis**.

# LudgerTag™ Glycopeptide Labelling & Enrichment Kit

2-hour sample preparation



V-Tag Glycopeptide Analysis Kit integrates with your **peptide mapping** workflow. It is used to **monitor batch-to-batch consistency** during the manufacturing of monoclonal antibodies (mAbs). Also, the kit complies with ICH Q2(R1) guidelines and can be used in comparability studies to **validate the biosimilarity** of new therapeutics.

LT-VTAG-24 allows the preparation of samples in **only 2 hours** starting with as little as **10µg** of mAb glycoprotein.

Proteolysis	Labelling	Clean Up	Analysis	Outcome
Trypsin Digest (1 hr)	Glycopeptide Labelling And Enrichment Kit <b>LT-VTAG-24</b> (1 hr)	LudgerClean A cartridges <b>LC-A-24*</b> (1 hr)	UHPLC and MALDI-MS	Reliable mAb glycoprofiling  Glycopeptide mapping

\* Glycopeptide enrichment cartridges (LC-A-24) can be purchased both individually and as part of the LT-VTAG-24 kit.

For more information about these products, please visit [our website](#) or [contact us](#).

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Should you wish to source our products locally, we have a global network of trusted and authorised distributors who will assist you in procuring our products. (Check our updated contact page for the [list of distributors](#) in different territories listed alphabetically).



If you have any technical or logistics questions? [Contact us directly](#). Our team of experts are here to help you find the perfect glycan analysis solution for your specific needs.

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