Ludger News

November/December 2023

Fast & reliable O-glycan release from glycotherapeutics

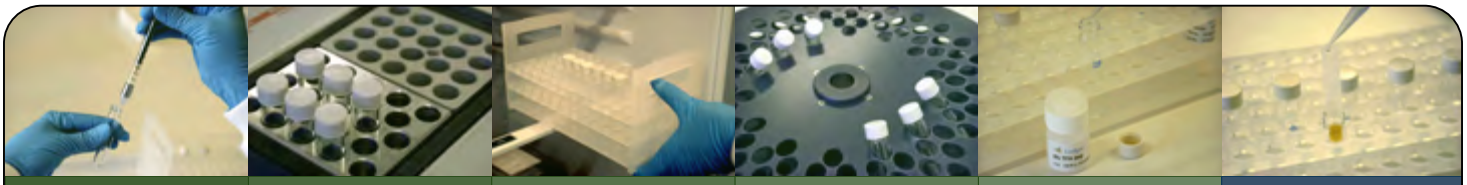
Without the harsh conditions of hydrazinolysis and the limitations of O-glycosidase

The **LudgerLiberate Orela Glycan Release Kit** (**LL-ORELA-A2**) is a novel product that uses a proprietary reagent to cleave O-linked glycans from glycoproteins in a **mild and selective manner**. The released glycans have **free-reducing termini** that allow fluorescent tagging by reductive amination. The kit contains reagents and materials for up to **12 samples** and can be used with biopharmaceutical glycoproteins of various types and sources.

The **advantages** of LudgerLiberate Orela Glycan Release Kit are as follows:

- It is **compatible with complex and sialylated O-linked glycans**, unlike O-glycosidase.
- It **preserves the native structure of the glycoprotein backbone**, unlike hydrazinolysis.
- It is **fast and easy to use**, requiring only a few hours of incubation and minimal sample preparation.

Follow the workflow below to **release the O-linked glycans in your therapeutic glycoprotein** sample.



Orela Reagent	Incubation	Cooling	Drying	Acidification	Clean-Up
Add 200 µl of Orela relase agent to a pure and dried sample	5-16 hrs @50°C	@Room Temperature	Centrifugal evaporation @30-40°C	200 µl of 50% Acetic Acid @4°C overnight	LudgerClean CEX cartridges

The resulting samples can then be **fluorescently labelled** and **analysed using HPLC**, **CE** or **MS**. If you require any further information, please contact us at **info@ludger.com**.

Christmas Orders and Delivery Information

Our offices will be closed between **December 25th** and **January 1st**.
Orders received before **December 15th** will be processed and delivered before Christmas.
First orders to go out in **2024** will be on **January 2nd**.

HPLC Columns Replacement Criteria and Care

HPLC columns are consumable items and as such over time you will begin to lose resolution of your critical pairs. There are some **signs that indicate when your HPLC column needs to be replaced**, such as:

Issue	Possible Cause	Recommended Solution
High or low pressure	Blockage or a leak in the column or the system	1) Check the pressure with and without the column to locate the source of the problem 2) Rinse, back flush, or change the column inlet frit to clear any clogs
Poor peak shape (distorted, split, or tailed)	Column contamination, degradation, or damage	1) Clean the column with suitable solvents or regenerate the column with stronger solvents. 2) Check the peak asymmetry and tailing factor to evaluate the peak shape
Low resolution	Loss of column efficiency or selectivity	1) Optimize the mobile phase composition, flow rate, temperature, or injection volume to improve the resolution 2) Check the height equivalent to a theoretical plate (HETP) and the number of theoretical plates to evaluate the column efficiency
Poor reproducibility (inconsitent retention time, peak area, or peak height)	Lack of column stability or consistency	1) Condition the column with several injections of sample or standard before running the method 2) Check the retention factor and system suitability test to evaluate the column performance

If none of these methods can restore your column performance to an acceptable level, you may need to replace your column with a new one. To extend your column lifetime, you should follow some good practices such as:

- **Use a guard column and an in-line filter** to protect your column from particulates and contaminants.
- **Wash your column before and after each use** with appropriate solvents to remove any residues or impurities.
- **Store your column in proper conditions** with suitable solvents and caps to prevent drying out or microbial growth.
- **Keep a log of column usage** to track the number of injections, mobile phases, samples, and methods used on your column

Glycoconjugates observed at the single-molecule level

Glycan characterization relies on a combination of mass spectrometry and NMR imaging to provide insights into size, sequence, branching and connectivity. However, determining the linkage positions and anomeric configuration of sugar residues in complex glycoconjugates remains a difficult task.

In the article **Direct observation of glycans bonded to proteins and lipids at the single-molecule level**, **Anggara et al.** demonstrated that glycoconjugates can be observed by direct imaging using low-temperature scanning tunnelling microscopy.

This technology could enable the direct observation of glycoconjugates beyond today's analytical capabilities. Please have a look at the image of **Ludger GPEP FA2 Glycopeptide Standard** obtained by the scientist (Figure 1).

Figure 1. STM imaging of single biantennary N-glycan NGA2F on the KVANKT peptide (GPEP-FA2-01)