News – March/April 2015

Sales orders can now be sent to **sales@ludger.com**

For general enquiries and quotations please contact us at **info@ludger.com**

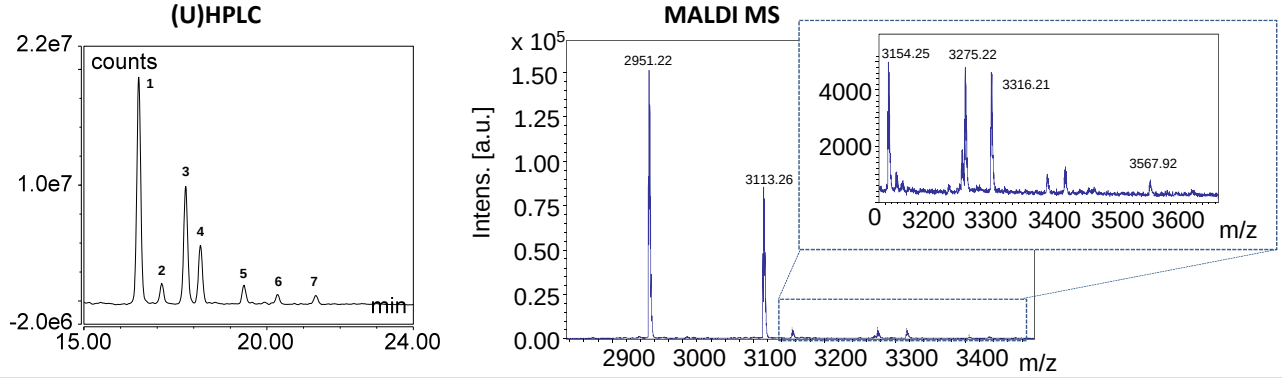
Ludger VTAG Glycopeptide Labeling and Enrichment kit

We are delighted to announce the launch of a kit for the labelling and enrichment of IgG glycopeptides, enabling analysis by (U)HPLC or MALDI Mass Spectrometry.

The VTAG kit (Cat # LT-VTAG-24) is suitable for IgG subclass glycoproteins that have been digested with pronase or trypsin to release the glycopeptides. As little as 5ug IgG sample can be used. The VTAG kit labels each sample in 1 hour and enriches the sample using a solid phase extraction (SPE) device for a further hour.

This kit has been validated according to ICH guideline Q2 (R1) guidelines. Using different IgG samples and replicates of 9 for each, CVs for repeatability were typically <5%.

Data: Example of IgG1



Ordering information:
Each kit is suitable for 24 samples. Cat # LT-VTAG-24

Procainamide labeled glycan standards

The following glycan standards have been labelled with procainamide and are available to order:

IgG glycan standard, 50pmol	Cat# CPROC-IGG-02
Man5 glycan standard, 20pmol	Cat# CPROC-MAN5-01
FA2G1 glycan standard, 20pmol	Cat# CPROC-FA2G1-01

Biopharmaceuticals With A High Content of Sialic Acid

Whilst monoclonal antibodies have little or no sialylation on their Fc portion, many more sialylated glycoproteins are regularly being developed by the biopharmaceutical industry. Examples include Fc fusion proteins, hormones (such as EPO), vaccines and clotting factors. In humans the major sialic acid on glycans is Neu5Ac, however Neu5Gc can be added to glycoproteins that are expressed in different cell lines. Since sialic acids can influence drug safety and efficacy, regulatory bodies are demanding more rigorous characterisation of glycans. With the widespread development of biosimilars by the industry, this has been highlighted by the new EMA Guideline on Biosimilars Quality which requires that extensive state-of-the-art characterisation studies are performed in parallel on both reference medicinal product and the biosimilar (see Reference below).

How can Ludger help?

There are a number of approaches that can be used to aid the characterisation of sialylated glycans using validated Ludger technology.

1. Identification and quantification of sialic acids:

a) Use the Ludger DMB Sialic Acid Labelling Kit (Cat# LT-KDMB-A1) to release, identify and obtain relative quantitation of Neu5Ac, Neu5Gc and Neu5,9Ac_2 by (U)HPLC analysis. Ludger's quantitative sialylated glycopeptide standard (Cat # BQ-GPEP-A2G2S2-10U) is recommended to check the efficiency of glycan release, labeling and recovery and will give you confidence in the accuracy of your sialic acid measurements.

b) Use of Ludger quantitative standards (Cat # CM-NEUAC-01, CM-NEUGC-01) to determine absolute amounts of Neu5Ac and Neu5Gc.

2. Analysis and characterisation of sialylated glycans:

N-glycans are removed from the drug by PNGase F (Cat # E-PNG-01) then fluorescently labelled with 2AA or 2AB using using LudgerTag kits incorporating 2-picoline borane reductant (Cat # LT-KAB-VP24 or LT-KAA-VP24) and cleaned up using LC-T1-A6 cartridges . A number of analyses can be performed:

- Analysis by HILIC- (U)HPLC to provide GU values and obtain preliminary structure assignments.
- Exoglycosidase sequencing can be performed to characterise the structures.
- Charge separation of labelled glycans can provide data on the relative proportions of the mono-, di-, tri- and tetra-sialylated glycans.
- Mass composition data can be obtained by (i) ESI-MS (and MS/MS) analysis of free glycans by direct infusion or LC-MS; or (ii) MALDI analysis following permethylation to stabilise the sialic acids.

A full report on these approaches is available on our website. If you require any further information on how we can help you select products (for your in-house analyses), Ludger services (our in-house analyses) or method transfer, please contact us at info@ludger.com

Reference: "EMA Revises Biosimilars Guideline on Quality Issues" in Hogan Lovells (ed) Focus on Regulation, posted on June 9, 2014 by Elisabethann Wright and Ciara Farrell.