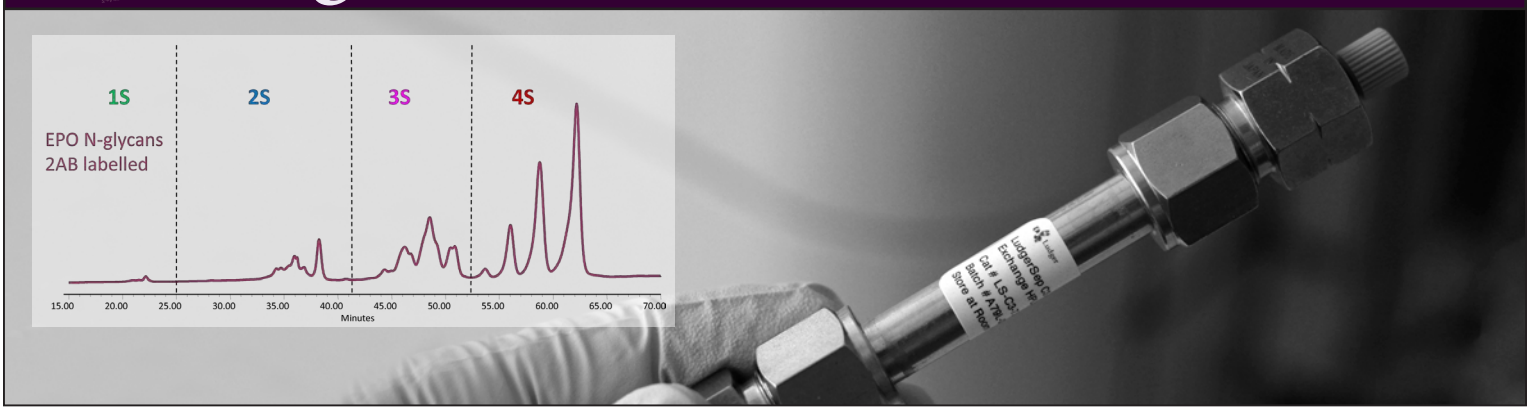
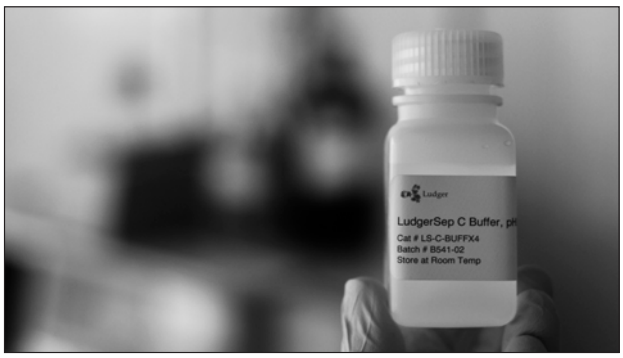
Ludger News

May/June 2017



Tools for Analysis of Negatively Charged Glycans



Negatively charged glycans (sialic acids, sulphated or phosphorylated sugars) often play a critical role in the function of a glycoprotein. For example sialic acids increase the serum half-life of glycoproteins by protecting them from degradation by the asialoglycoprotein receptor; sulphated glycans are involved in cell adhesion; and Mannose-6-Phosphate is a key targeting signal for transport of glycoproteins to lysosomes and is present in therapeutic enzymes (enzyme replacement therapies) developed for treatment of lysosomal storage diseases.

The **LudgerSep-C3 column** (Cat # LS-C3-7.5x75) is a weak anionic exchange (WAX) HPLC column that enables you analyse negatively charged sialylated, phosphorylated and sulphated glycans. This technique is also known as 'charge profiling'. An example of the information that can be provided is the relative amounts of sialylation (1, 2, 3 or 4 sialic acids) on your glycoprotein which is important to know when analysing a highly sialylated protein such as erythropoietin (EPO).

Although sialylated and sulphated glycans can be separated by anion exchange at a low pH of 4.4, the phosphorylated sugars would not be fully charged and there would be multiple species in solution. In order to have one buffer which is suitable for separation of all anionic glycans (sialic acids, phosphorylated and sulphated sugars) Ludger recommends the use of pH9 ammonium formate buffer. The **LudgerSep C buffer** is a pH 9 ammonium formate buffer concentrate (Cat# LS-C-BUFFX4) which is suitable for separation of all anionic glycans (sialic acids, phosphorylated and sulphated sugars). This concentrate can easily be diluted with water and acetonitrile then used directly as a solvent for the LudgerSep C3 column.

Product Info:

LudgerSep-C3 column	Cat # LS-C3-7.5x75
LudgerSep C buffer concentrate	Cat # LS-C-BUFFX4

For enquiries or more information, please contact info@ludger.com

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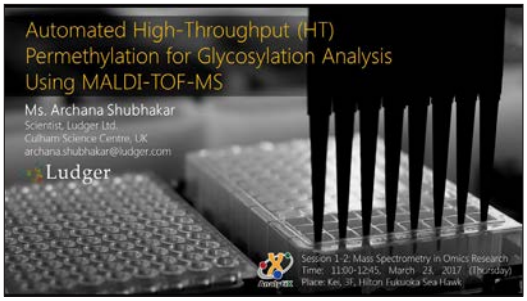
High-Throughput Permethylation presentation at Analytix 2017, Japan



Archana (Mili) Shubhakar was invited to give a presentation at "BIT's 5th Annual Conference of AnalytiX 2017 (emerging trends in Analytical Science)" on March 22-24, 2017 in Fukuoka, Japan.

For more information on permethylation and MALDI-TOF-MS please contact us or visit the following page of our website:
www.ludger.com/permethylation

An Analytical Approach to Studying Monosaccharides:

Quantitative Monosaccharide Analysis is a requirement in drug development as it falls within ICH guidelines Q6B and Q5E for comparability studies during product development and after major manufacturing changes. The **LudgerTag monosaccharide release and labelling kit** (LT-MONO-96) is designed to release monosaccharides from a glycoprotein. The released monosaccharides have a free reducing terminus to allow fluorescent labelling with 2-aminobenzoic acid (2-AA) by reductive amination. Samples can then be analysed with (U)HPLC.

The absolute amounts of monosaccharides *N*-acetylglucosamine (GlcNAc); *N*-acetylgalactosamine (GalNAc); galactose (Gal), mannose (Man), glucose (Glc) and fucose (Fuc) can then be calculated by reference to standard curves from Mono-Mix quantitative standard (which is provided with the kit). Quantitation of these monosaccharides can be expressed as nmoles/mg or moles/mole protein.

When analysing a glycoprotein for the first time we recommend using both acids (2M TFA and 6M HCl) to remove monosaccharides from the glycoprotein: 2M trifluoroacetic acid (TFA) is sufficient to release the neutral monosaccharides from a glycoprotein. 6M hydrochloric acid (HCl) is a stronger acid which is more effective with GlcNAc and GalNAc monosaccharides. A side effect of using the stronger acid is that decomposition of the hexose sugars can occur, but it is important to compare results for both release methods to find the most appropriate acid release method.

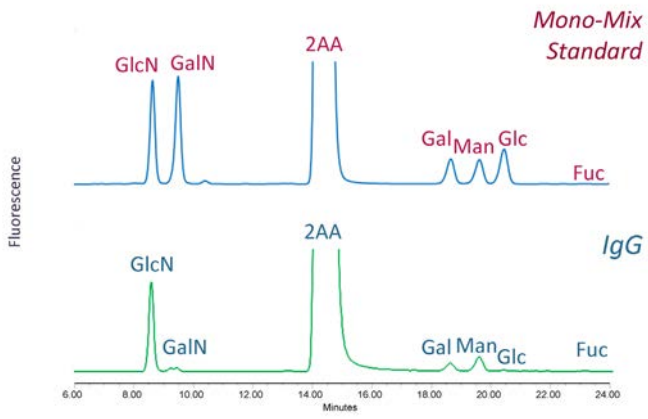
Here are two ways to get started with monosaccharide analysis:

1. We can provide analytical services for monosaccharide analysis and transfer the methods to your labs
2. You can order the LT-MONO-96 kit from Ludger and start using it straight away

The example below gives representative data for monosaccharides released using TFA and labelled with 2AA. Here we compare the Monomix (provided with the kit) to a human IgG (Ludger product GCP-IGG) sample:

Results

- Quantitation of monosaccharides as nmoles/mg protein



Monosaccharides quantified in nmoles/mg protein by reference to standard curves

TFA Release	nmoles/mg protein	
Monosaccharide	Average	%CV
GlcN	47	1.7
GalN	8	0.5
Gal	24	2.4
Man	41	1.6
Glc	5	9.2
Fuc	6	1.3