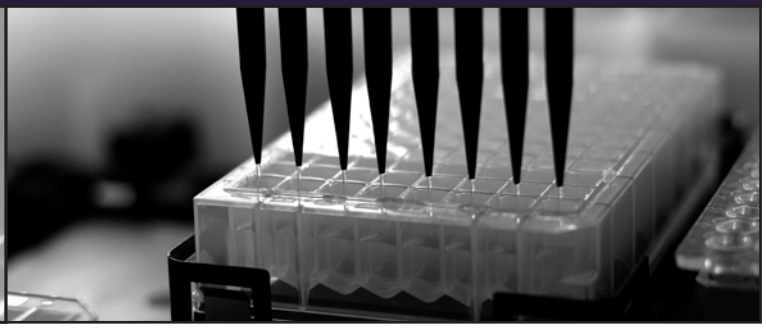
Ludger News

January/February 2016



Permethylation of Glycans Using Ludger Technology

Permethylation is a popular technique for the derivatisation of carbohydrates for MALDI-MS detection, as it enhances ionization efficiency, stabilizes the sialic acids and aids linkage analysis studies. On average one sample can take as little as 1 minute for data acquisition, making it a useful method for QbD and biomarker studies. Because conventional in-solution glycan permethylation is labour intensive with long turnaround times, we have developed a microplate based kit which is cost effective, automated and high throughput. The new kit, **LT-PERMET-96**, can be used to process 1-96 samples using either a manual method or an automated method that has been adapted to a liquid handling robot. Our new workflow consists of the following steps (i) automated glycan N-glycan release and (ii) automated enrichment of released N-glycans using LudgerClean™ pre-permethylation plate- **LC-PERMET-96**, (iii) automated HT permethylation of released glycans (N- and O- glycans),(iv) automated liquid-liquid extraction (v) automated data acquisition of permethylated glycans using MALDI-MS and (vi) Semi-automated data analysis.

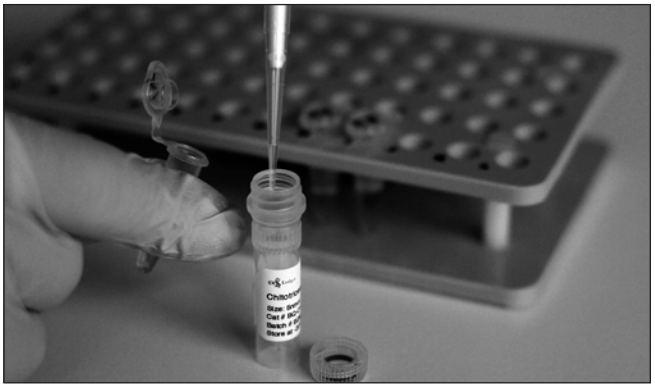
LT-PERMET-96 gives excellent signal enhancement due to increased ionization efficiency and the technology has been validated according to ICH Q2 (R1) guidelines (for Analytical Validation). Intra assay repeatability CVs for relative % intensities were <12% for major N-glycans from human IgG with a relative % areas of ≥ 5%.The results from validation studies suggest that this permethylation technique gives data that is comparable to UHPLC for 2-AB labelled and procainamide labelled glycans.

For more information regarding this technology please contact info@ludger.com

---

Bioquant Chitotriose Standards for reliable glycan quantification

Our quantitative chitotriose standard (BQ-CHITOTRIOSE-01) can be easily incorporated into your **2-AA or 2-AB glycan labeling workflow** to give you reliable glycan quantification and identification. Data is comparable to gold-standard glycoprofiling methods.



Here is our 4 step method:

1. Transfer your glycan sample into Chitotriose standard vial
2. Fluorescently label the Chitotriose-sample mixture (using LT-KAB-A2 or LT-KAA-A2 kits)
3. Clean up samples using LC-S-A6 or LC-T1-A6
4. Run on HPLC/UHPLC

The procedure is compatible with 96-well plate based assays, enabling high-throughput studies using a liquid handling robot.

Until March 1st 2016 receive 10% discount on orders of BQ-CHITOTRIOSE-01 when you use the following code with your order: CHITO10

High throughput mAb analysis service

Our GX-mAb service provides parallel analysis of hundreds of mAb samples at an affordable cost. You can select the analytical platform that you would like us to use giving you information on glycan relative quantitation and identification. Results are typically within two weeks of starting sample analysis.

For more information visit www.ludger.com/glycan-analysis-services/ or contact Richard Gardner: richard.gardner@ludger.com.

Hydrazine

This is now available from Ludger.
Contact us on info@ludger.com for pricing.

Ludger Clean EB10 Cartridges for Mass Spectrometry

The use of Mass Spectrometry (MS) to analyse biopharmaceuticals can be enhanced with the use of LudgerClean EB10 cartridges. The sample analysed contains 5µg of 2AA labelled glycans released from human IgG antibody glycoprotein. Originally in 0.01M PBS buffer the sample was de-salted using an EB10 cartridge and analysed by MALDI-MS.

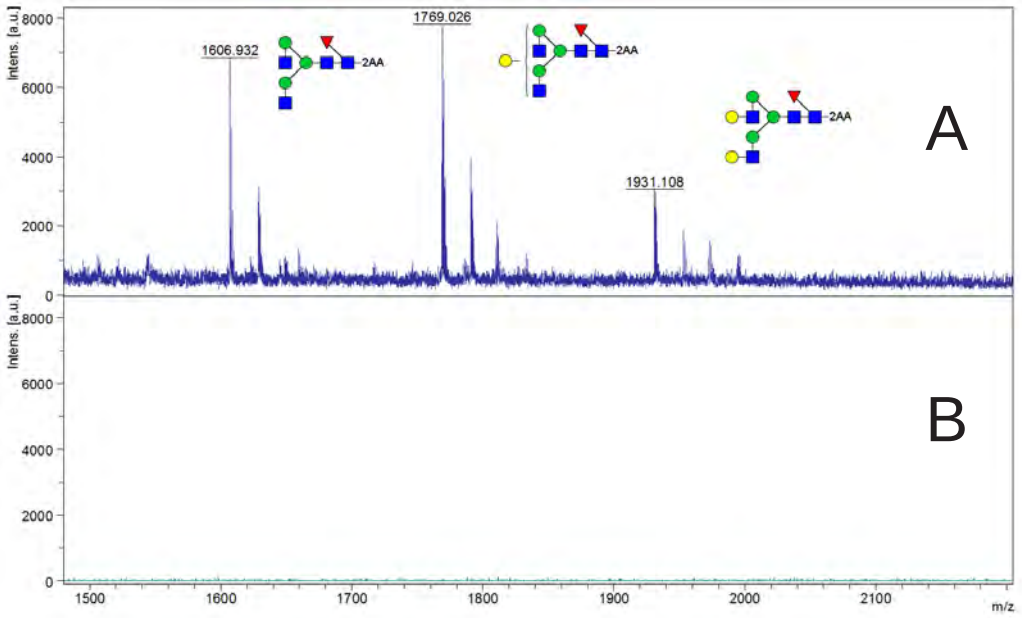


Figure 1: Trace A shows the glycans following clean up with EB10 cartridges the glycan peaks can clearly be seen (with the structures of the most prevalent peaks indicated). Trace B shows the sample before de-salting in which the PBS buffer completely suppresses the signal.

Sialate-O-acetylesterase, SIAE

You can now order sialate-O-acetylesterase (SIAE) in a kit from Ludger. This enzyme (also known as sialic acid acetyl esterase and Nan S) is a recombinant protein from Tannerella forsythia, ATCC 43037 strain, expressed in Escherichia coli. The enzyme removes extra acetyl groups attached via an O- group from sialic acids, e.g. acetyl groups in the 9, 8 or 7 positions. The amount and distribution of these extra sialic acid acetyl groups may influence the effectiveness of biopharmaceuticals such as erythropoietin (EPO) and could vary from one pharmaceutical production batch to another, making them a potential critical quality attribute (CQA) to be routinely monitored.

Sialate-O-acetylesterase can act upon complex glycoprotein samples such as EPO, bovine submaxillary mucin and oral epithelial cell-bound glycans, and on N- and O-glycans released from a glycoprotein. Either fluorescently-labelled or unlabelled glycans are suitable. It can also be used for free sialic acid samples (i.e when using our DMB kit), to help identify them.

The kit, containing enzyme and reaction buffer (Cat # **LZ-ACASE-KIT**), is sufficient for up to 50 samples.

Biochem J. 2015 Dec 1;472(2):157-67. doi: 10.1042/BJ20150388. Epub 2015 Sep 16.
Characterization of a sialate-O-acetylesterase (NanS) from the oral pathogen Tannerella forsythia that enhances sialic acid release by NanH, its cognate sialidase.
Phansopa C[1], Kozak RP[2], Liew LP[2], Frey AM[1], Farmilo T[1], Parker JL[1], Kelly DJ[3], Emery RJ[2], Thomson RI[4], Royle L[2], Gardner RA[2], Spencer DI[2], Stafford GP[5]