Ludger News

March/April 2021

2-picoline borane (2PB) - 'The safer reductant'

Available with these LudgerTag Labelling kits:

LT-KAB-VP24 2-AB glycan labelling kit (2PB reductant)
LT-KAB-VP96 2-AB glycan high throughput labelling kit (2PB reductant, 96 samples)
LT-KAA-VP24 2-AA glycan labelling kit (2PB reductant)
LT-KPROC-VP24 Procainamide glycan labelling kit (2PB reductant)

Ludger Glycoanalysis-Tools: labelling kits containing the non-toxic reducing agent 2-picoline borane (2PB)

Fluorescent labelling methods for glycoanalysis of therapeutic glycoproteins are well established and aid subsequent separation and quantitation of glycans using a range of techniques. These include high performance liquid chromatography (HPLC and UHPLC), capillary electrophoresis (CE), and mass spectrometry or a combination thereof. Ludger provides a range of labelling kits to fluorescently label N- and O-glycans, that have been released enzymatically or chemically. These include 2-aminobenzamide (2AB) labelling, procainamide (PROC) labelling and 2-aminobenzoic acid (2AA) labelling kits.

Many commercially available 2AB, PROC and 2AA labelling kits use sodium cyanoborohydride as the reducing agent during glycan labelling. Although this reductant is a gold standard it is toxic and therefore the best practice is to perform the labelling in a fume cupboard for safe handling. To provide a safer, less toxic option that can be used on a laboratory bench without the need to use a fumehood, Ludger offers labelling kits that use 2-picoline borane (2PB), which is a significantly safer reductant.

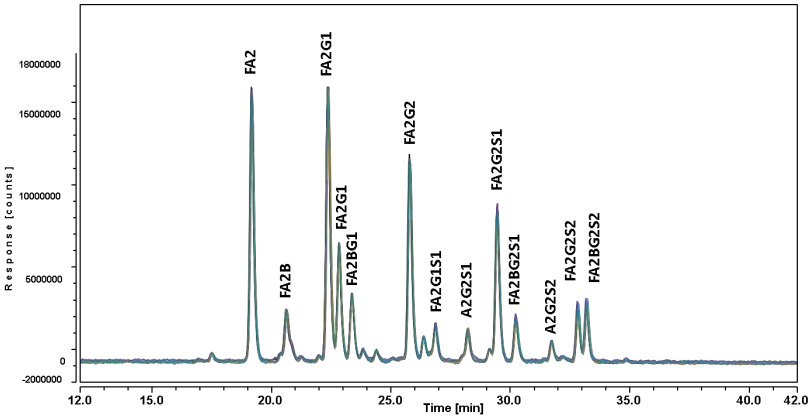


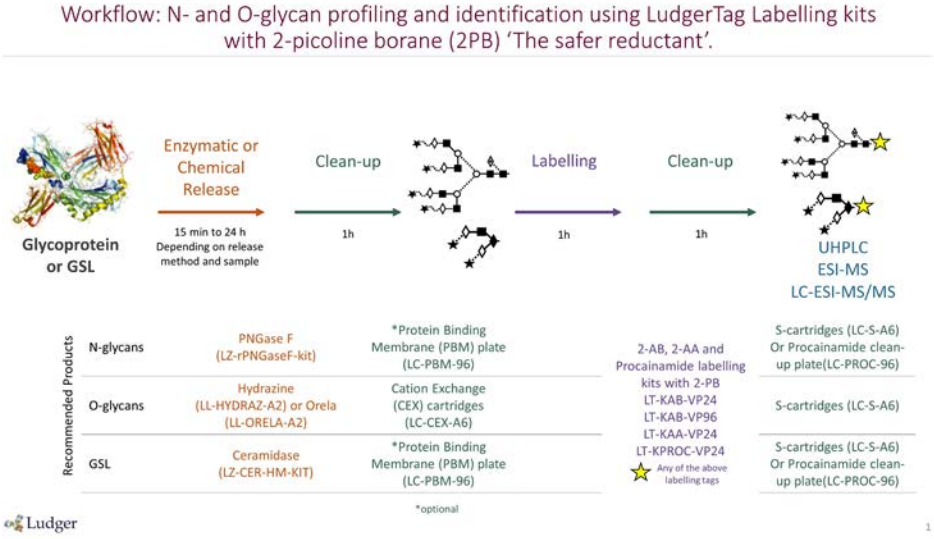
Figure 1: Overlay of 8 human IgG N-glycan samples analysed using a HILIC UHPLC column: four samples labelled using the procainamide labelling kit with 2PB as the reductant and four samples labelled using the procainamide labelling kit with sodium cyanoborohydride as the reductant

Features and benefits of LudgerTag™ 2PB kits

The labelling kits containing 2PB as the reductant are equally efficient with respect to glycan labelling performance as the traditional sodium cyanoborohydride kits (See **Figure 1**, a typical chromatogram overlay of PROC labelled human IgG N-glycans).

Fully validated Ludger labelling kits

The 2PB containing kits have been validated following ICH Q2(R1) guidelines. Precision values are excellent, with coefficient of variation (CV's) of less than 5% for peaks with relative % areas greater than 5% and with CVs of less than 8% for peaks with relative % areas less than 5%. The kits offer the equivalent labelling efficiency as the standard Ludger Tag 2AA, 2AB and PROC labelling kits containing sodium cyanoborohydride.



Improved safety

2PB has a lower toxicity compared to sodium cyanoborohydride and therefore is safer.

Ease of use

Each kit comprises three bottles or vials; one containing acetic acid and DMSO solution, the second vial has the reductant 2PB and the third vial has the dye (2AB, 2AA or PROC). As there are fewer vials involved in the make- up of the labelling solution, it makes the labelling process simpler and more efficient.

For more information on our labelling kits containing 2PB as the reductant please visit: our products page for Glycan Labelling and Derivatisation Kits. For enquiries or more information, please contact: info@ludger.com

Publication in PLOS Neglected Tropical Diseases: Tsetse salivary glycoproteins are modified with paucimannosidic N-glycans, are recognised by C-type lectins and bind to trypanosomes

Photo credit: Oregon State University © 2011

Successful collaboration between Ludger and The Liverpool School of Tropical Medicine, resulted in publishing an article in PLOS Neglected Tropical Diseases titled "*Tsetse salivary glycoproteins are modified with paucimannosidic N-glycans, are recognised by C-type lectins and bind to trypanosomes.*"

African sleeping sickness is caused by Trypanosoma brucei, a parasite transmitted by the bite of a tsetse fly. Most research has focused on the salivary proteins, while glycans that modify them remain unexplored. Insect salivary glycans may affect how the saliva is recognized by the host, possibly playing a role during pathogen transmission. This is the first study of the salivary glycans of tsetse fly Glossina morsitans providing detailed information about their structures and relative abundances. This study shows that tsetse fly glycoproteins are mainly modified by simple N-glycans with short mannose modifications, which are recognised by mammalian C-type lectins (mannose receptor and DC-SIGN). This information provokes interesting questions as to the role of these glycoproteins in the successful establishment of infection by this parasite.

Ludger's contribution to this study included:

- Help with the study design and strategy
- Release of N- and O-glycans from tsetse fly saliva
- N- and O-glycan profiling and characterisation using orthogonal techniques such as UHPLC, LC-ESI-MS and exoglycosidase digestions (see **Figure 1** for data on salivary N-glycans from Tsetse flies, before and after digestion with exoglycosidases)
- Supporting the visual reporting of structures and nomenclature of glycans identified in the study

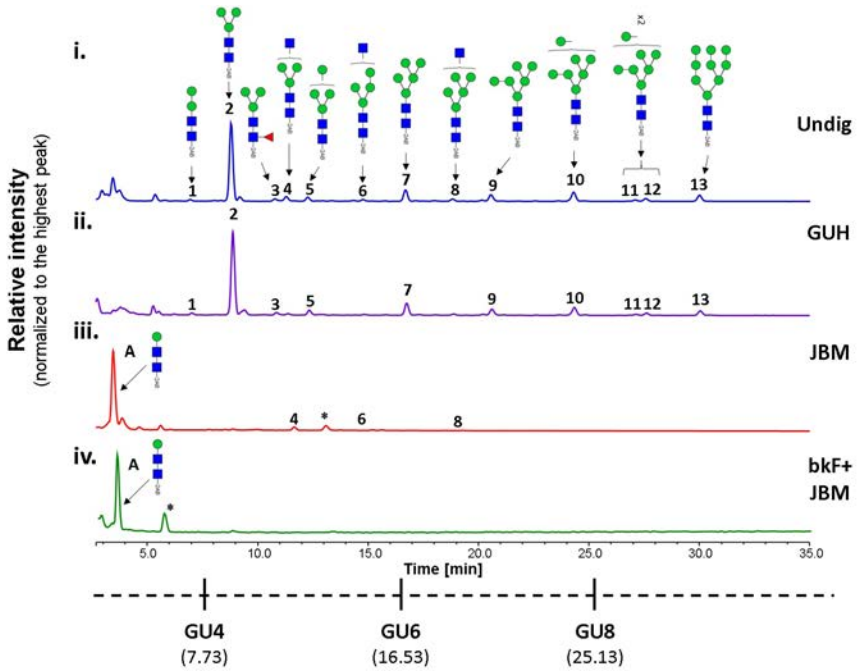


Figure 1: Profile of salivary N-glycans from teneral (young, unfed) flies, before and after digestion with exoglycosidases. Aliquots of the total PNGase F-released 2-AB-labeled N-glycan pool were either undigested (i) or incubated with a range of exoglycosidases (ii-iv). (i) Undig, before digestion; (ii) GUH, Streptococcus pneumoniae in E. coli β-N-acetylglucosaminidase; (iii) JBM, Jack bean α-Mannosidase; (iv) bkF, Bovine kidney α-fucosidase. Following digestion, the products were analyzed by HILIC-UHPLC.

To find out how to utilise enzymes in glycan characterisation visit our Exoglycosidase enzyme page. Visit our Procainamide webpage for more information on how to characterise glycans using LC-MS. And for more information about this article visit our Publications webpage.