

News – September/October 2014

Glycan release, labelling and clean up using a liquid handling robot

We have now added another step to our Ludger VP-T1 system on the Hamilton Microlab STARlet liquid handling robot. By incorporating the initial enzyme release step into the process, the system now takes up to 96 glycoprotein samples from N-glycan release through to labelling and clean up and preparation of labelled N-glycan samples ready for analysis on HPLC/UPLC.

The work flow is a five-step process and the total time taken is 50 hours:

- 1. In solution PNGase F release of N-glycans from glycoproteins
- 2. Acidification and purification of released N-glycans
- 3. Labelling of N-glycans with 2AB or 2AA using LudgerTag VP kits
- 4. SPE sample clean-up using LudgerClean T1 cartridges
- 5. Preparation of samples for HPLC/UPLC analysis

Validation: This process has been validated here at Ludger using 48 human IgG and 48 bovine fetuin samples. This method was shown to have good repeatability; intra assay **CVs of <0.5% on GU values** and **CVs of <5% on peak areas** for glycan peaks with an average relative % area >1.0% (48 samples). Shown below in Figure 1 are overlaid UHPLC chromatograms from the analysis of 48 samples of human IgG:

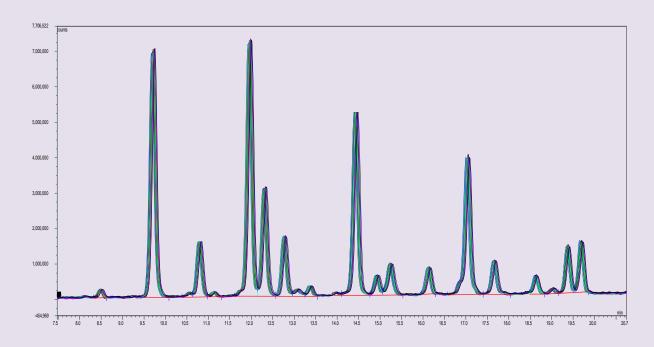


Figure 1: Overlay chromatogram of 48 UHPLC analyses of human IgG N-glycan. Y axis normalised to highest peak. The CVs on GU values were less than 0.5 % across all 21 IgG peaks from 48 UHPLC analyses. The CVs on average % areas were less than 5 % on 14 of the 21 peaks (all peaks with average % areas above 1 %)

LudgerTag VP96 kits

Manufacture of our 96-sample format kits for labelling glycans with 2AB or 2AA is underway. These kits are compatible with a liquid handling robot and will soon be available.

Using 2-picoline borane (PB) as a reductant, these kits are a higher throughput version of our LT-KAB-VP24 and LT-KAA-VP24 kits.

Product:	Cat No.
LudgerTag 2AB labelling kit for 96 samples (PB reductant)	LT-KAB-VP96
LudgerTag 2AA labelling kit for 96 samples (PB reductant)	LT-KAA-VP96

Ludger in China

We are pleased to welcome Lily Wang to our team in Shanghai. Lily is Ludger's Technical Sales Executive in China. Together with Cindy Li (Sales Executive, Ludger China) she will be assisting our clients in China with technical questions relating to our products and services.

Shanghai office:

Plaza 66, 15/F, Tower 2 1266 West Nanjing Road Jing'An District Shanghai 200040 China

中国上海 静安区南京西路1266号 恒 隆广场2期15楼200040

Telephone: +86-02160398391

For all enquiries please contact us via e-mail at <u>info@ludger.com</u>

Need help with glycan release and labelling?

The technical aspects of sample preparation can be complicated. We can help you to get your manual method for glycan release and labelling up and running. Once you have ordered the following products we can send you the methods we use in house:

Product:	Cat No.
PNGase F for glycan release	E-PNG-01 or E-PNG-05
Post release PBM clean up plate	LC-PBM-96
VP24 labeling kits for 2AB or 2AA	LT-KAB-VP24 or LT-KAA-VP24
Post labellingT1 clean up cartridges	LC-T1-A6

If you would like a quotation please let us know.