News

Please contact us if you wish to receive information about Ludger products, request a quote, place an order or to enquire about our custom glycoprofiling services:

Contact us: info@ludger.com

New format VP kits

Following studies at Ludger, our LudgerTag VP24 kits using 2-picoline borane (2PB) now comprise the following three reagents (2 sets of reagents per kit):

Dye (2-AB or 2-AA)
2PB reductant
Acetic acid / dimethyl sulfoxide (DMSO) solution

The labelling efficiency is the same as the previous format. Preparation of the labelling reagent is easy to do: just add the acetic acid/DMSO solution to the ampoule of dye and mix until the dye is fully dissolved, then add this solution to the ampoule of 2-PB reductant. Once fully dissolved the labelling reagent is ready to be added to your samples.

Ludger V-tag: a fluorescent label for glycopeptides suitable for UHPLC or MALDI mass spectrometry

Ludger's V-tag kits (Cat # **LT-VTAG-24**) for labelling IgG glycopeptides give you the flexibility of analysing your samples by UHPLC or MALDI mass spectrometry. The method involves the digestion of glycoproteins with pronase followed by labelling with V-tag and the enrichment using SPE cartridges. As little as 10ug IgG sample can be used. There is no need for drying down samples before labelling and the process can be performed within one working day. This method can be also performed using a liquid handling robot. V-tag kits are suitable for 24 samples.

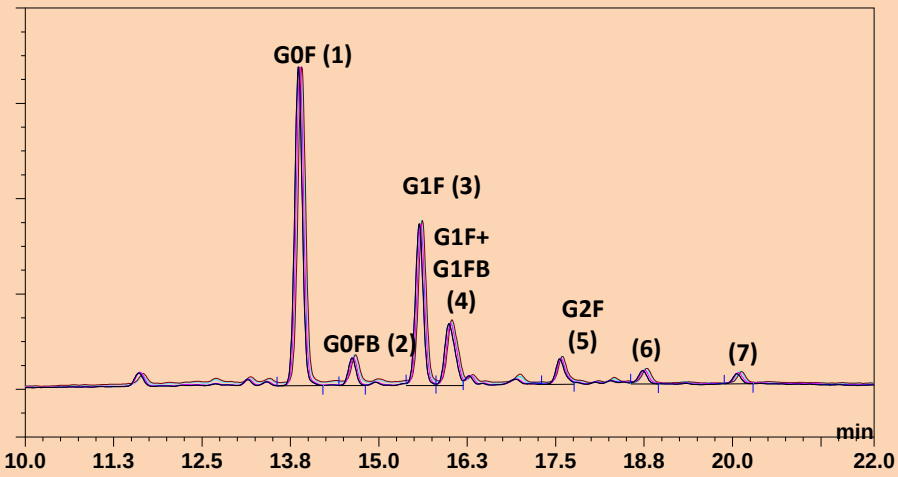

Figure 1: BEH UHPLC profile of V-tag-IgG-1-labelled glycopeptides. 6 replicates are overlaid here. CVs were < 6 %. Three replicates were performed on Day 1 and 3 replicates performed on Day 2; Intermediate precision

Releasing O glycans without hydrazinolysis

Biotherapeutics can contain O-glycans and it is important to monitor this during drug development. As a first step in quantitative analysis of O-linked glycans they must be released from glycoproteins in high yield, nonselectively, unmodified, and with a free reducing terminus so that they can be fluorescently labelled.

Since no enzyme has yet been ìsolated for the universal release of O-glycans from glycoproteins, chemical methods are used; either hydrazinolysis or non-reductive β-elimination. Ludger sells kits for both methods; a hydrazinolysis kit (Cat # LL-HYDRAZ-A2) and a kit for non-reductive beta elimination, the ORela kit (Cat #LL-ORELA-A2).

Unfortunately all of the chemical methods for O-glycan release show degradation (the loss of monosaccharides from the reducing terminus of the glycans known as “peeling”). It results in poor repeatability and stoichiometry for analytical glycan profiles. The mechanism for formation of this unwanted degradation product is not fully understood.

Whilst hydrazinolysis is considered the most effective method for O-glycan release in terms of yield and peeling, hydrazine is highly toxic so must be handled in in a dry, anaerobic environment . The reagents used for the non-reductive β-elimination method are safer and much easier to handle but peeling can be a problem.

Ludger is developing an improved β-elimination method that releases O-glycans with low levels of peeling (i.e. comparible to those seen when using hydrazinolysis). Typical results are shown in Figure 1.

This method will be available in a kit format soon. If you are interested in finding out more please contact us.

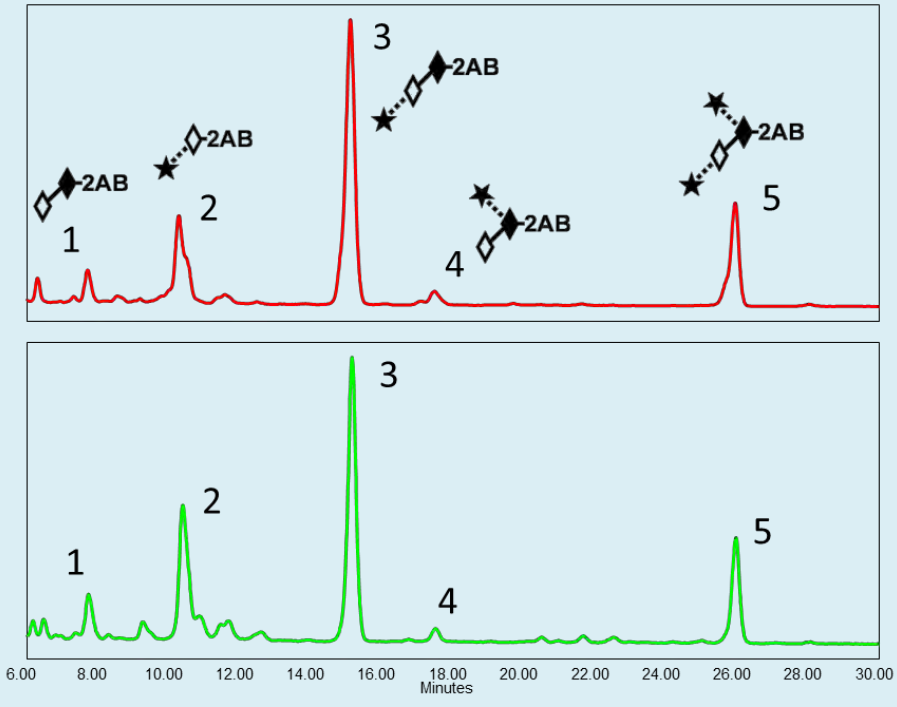

Figure 2: Analysis of O-glycans released from EPO. Two O-glycan release methods were used Hydrazine (top chromatogram) vs beta elimination (LudgerLiberate Orela kit; bottom chromatogram). Peak 2 is the peeled product