

Ludger's Custom Analytical Services --  
Characterising Glycans from SARS-CoV-2  
Specimen Samples and Vaccine Candidates

## Characterising Glycans from SARS-CoV-2 Specimen Samples and Vaccine Candidates using Ludger's Custom Analytical Services

The current global situation with Coronavirus disease 2019 (COVID-19) has changed our daily lives and the way we interact.

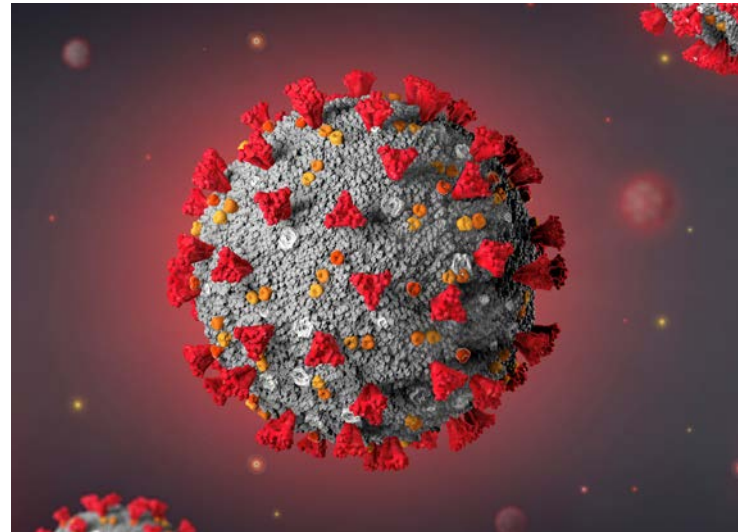
The COVID-19 is caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). Understanding the N-glycosylation of the SARS-CoV-2 viral spike protein variations and its potential effects on the interaction with host immune system is essential in the vaccine development and for understanding the disease progression.

Ludger offers custom analytical services to suit your individual requirements. We have many years of expertise with analysing glycosylation (including N- and O- glycosylation) from variety of sample types and our laboratories are prepared to receive:

- COVID-19 patient samples (e.g. plasma, tissues)
- SARS-CoV-2 infected cell lines
- Vaccine candidates
- mAbs, glycoprotein hormones, Fc fusion proteins

For more information, please visit the [Glycan Analysis Services](#) pages.

If you have any questions relating to glycan analysis, or to request a Study Proposal and/or quotation, please contact us at [info@ludger.com](mailto:info@ludger.com) or contact Dr Radoslaw Kozak (Head of Glycoprofiling, Ludger) at [rad.kozak@ludger.com](mailto:rad.kozak@ludger.com)



## Ludger Application Note: O-glycosylation analysis

**O-glycosylation analysis**  
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**Why is O-glycosylation analysis important?**  
Protein O-glycosylation is one of the most common types of post-translational modifications. It refers to the covalent attachment of a carbohydrate moiety to the polypeptide backbone. In O-link glycosylation the glycan is attached to the side chain of a Ser or Thr residue.

O-glycosylation is known to have a critical impact on protein secretion and protective immunity against cancer, and maintenance of normal development and physiology. Moreover, a substantial portion of the protein-based pharmaceuticals on the market are glycoproteins where O-glycosylation has found to critically modulate the physicochemical properties of proteins, have a functional impact on their therapeutic potentials, and affect the safety profiles of such drugs. O-glycosylation is prevalent in many classes of therapeutic proteins including Erythropoietin (EPO), follicle stimulating hormone (FSH), Etanercept, Granulocyte-colony stimulating factor (G-CSF) providing evidence of its critical involvement in drug performance and diseases.

In-depth understanding of the O-glycosylation status of these drug substances will elucidate the structure-function relationship of the O-linked sugars, which may lead to the identification of functionally favorable O-glycan structures to improve drug efficacy and safety profile.

**Challenges**  
O-linked glycans can vary widely in size, ranging from a single N-acetylgalactosamine (GalNAc) monosaccharide at the reducing end and terminate to large oligosaccharides exhibiting complex glycan motifs resulting from further modifications by the addition of other sugar types including galactose (Gal), N-acetylglucosamine (GlcNAc), GlcNAc, fucose (Fuc), and sialic acids (Neu5Ac and/or Neu5Gc). This substantial heterogeneity can generate eight main core structures, each potentially subject to further elongation and substitution (Figure 1).

**O-GalNAc glycan cores**

Core 1: GalNAc, GlcNAc, Galactose  
Core 2: GalNAc, GlcNAc, Galactose, GlcNAc  
Core 3: GalNAc, GlcNAc, Galactose, GlcNAc, GlcNAc  
Core 4: GalNAc, GlcNAc, Galactose, GlcNAc, GlcNAc, Galactose  
Core 5: GalNAc, GlcNAc, Galactose, GlcNAc, Galactose, GlcNAc  
Core 6: GalNAc, GlcNAc, Galactose, GlcNAc, Galactose, GlcNAc, Galactose  
Core 7: GalNAc, GlcNAc, Galactose, GlcNAc, Galactose, GlcNAc, Galactose, GlcNAc  
Core 8: GalNAc, GlcNAc, Galactose, GlcNAc, Galactose, GlcNAc, Galactose, GlcNAc, Galactose

Legend: GalNAc (yellow), GlcNAc (blue), Galactose (green)

Figure 1. Eight main core O-glycan structures.

The analysis of O-linked glycosylation remains a challenge due to the lack of equivalent enzymes and the inherent structural heterogeneity of O-glycans. The specificities of the reported O-glycanase enzymes are predominantly restricted to T antigen (Galβ1,3-GalNAc-6-Ser/Thr). Despite limited reports describing O-glycosidases of broader specificities, a universal O-glycanase that is capable of liberating O-glycans with more sophisticated branching and elongation is still unknown. Therefore, the release of O-linked oligosaccharides is commonly achieved by chemical approaches where a broad range of samples, including but not limited to glycoprotein therapeutics, mammalian cell lines and bacterial cell components, biological fluids and tissues, can be processed.

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Our application note provides the details on how to perform O-glycan analysis using Ludger's glyco-analytical technology which includes:

- **O-glycan release:** by using Ludger enzyme (E-G001) for release of unsubstituted Galβ1,3-GalNAcα disaccharides and/or Ludger Liberate Hydrazinolysis (LL-HYDRAZ-A2) and Orela (LL-ORELA-A2) kits for complete removal of O-glycans from glycoproteins or glycopeptides.
- **O-glycan derivatisation**
- **Use of system suitability standards**

To enquire or place an order please contact: [info@ludger.com](mailto:info@ludger.com)

For more information and to view this and our other application notes, please visit our [Support-Resources](#) webpages.

## Publication in Glycoconjugate Journal: A novel glycosidase plate-based assay for the quantification of galactosylation and sialylation on human IgG

Ludger is pleased to announce the publication in the Glycoconjugate Journal of a scientific paper written by Osmond Rebello who was one of our Marie Skłodowska Curie early stage researchers on the EU Horizon 2020 funded project GlySign ([www.glysign.eu](http://www.glysign.eu)). This paper explores the use of a microtitre plate based assay for assessing the galactose levels present in protein G extracted IgG from patient blood samples and comparing the amounts detected with a higher end LC-MS technique. The plate based assay contains an exoglycosidase enzyme that was developed by our Innovate UK/BBSRC IBCatalyst funded collaborators Drs Lucy Crouch and David Bolam as part of the Glycoenzymes for Bioindustries project ([www.glycoenzymes.com](http://www.glycoenzymes.com)).

Immunoglobulin IgG circulating in people is a glycosylated protein which contains a single glycan per Fc region. This glycan lends itself to bioactivities within people with the level of galactosylation being linked to inflammation mechanisms of the immune system. Lower levels of IgG galactosylation are seen in patients with diseases of inflammation such as rheumatoid arthritis and inflammatory bowel disease as well as in people with high levels of chronic inflammation and those of advanced age, known as “inflammaging”. Currently technique for measuring the galactose levels will involve high-end instrumentation such as UHPLC and mass spectrometry. This paper explored the use of a simple plate based assay for measuring galactose and compared this favourable to the results using UHPLC/HILIC. Once prepared galactose levels can be determined for hundreds of samples within minutes.

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](#).

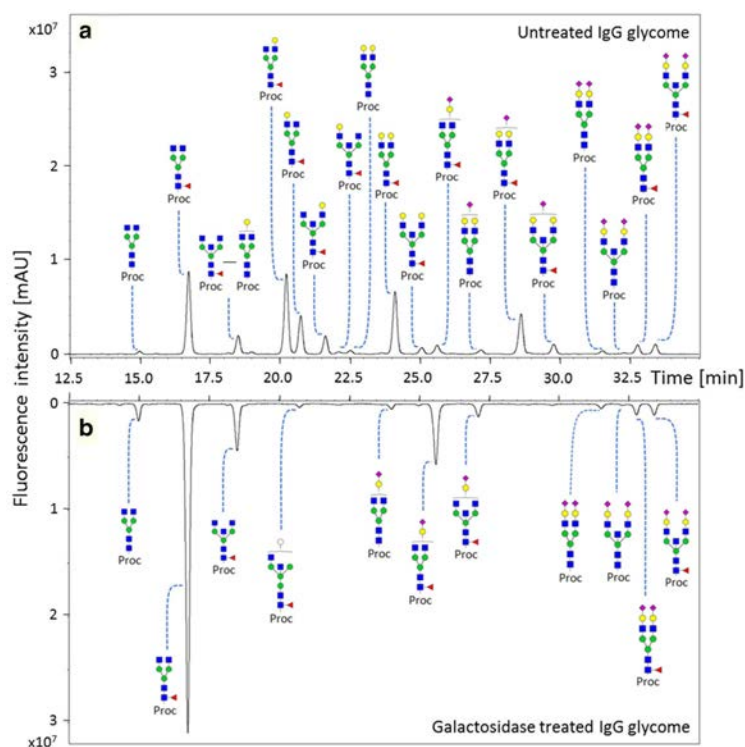


Fig 1. The released N-glycans from IgG glycoproteins (a) without galactosidase treatment was compared with (b) galactosidase treatment on a HILIC-FLD-MSn platform after labelling the reducing end with procainamide

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