

Alpha-Gal-containing biologics

Kozak RP -- Ludger Ltd, Culham Science Centre, Oxfordshire, UK

Glycans containing the non-human epitope Gal α 1-3Gal (alpha-gal) can significantly decrease the clinical performance of therapeutic monoclonal antibodies (mAbs). The presence of Gal α 1-3Gal can affect the safety profile and lead to a potential adverse reaction and neutralisation of the drug by anti- α -galactose antibodies reducing therapeutic efficacy. Given the potential impact on patients, Gal α 1-3Gal are a high priority Glycosylation Critical Quality Attribute (GCQA) and drug developers must effectively optimise, measure and control the glycosylation of their products to limit Gal α 1-3Gal levels throughout the product life cycle.

Detecting and quantifying the amounts of Gal α 1-3Gal can be very difficult as these epitopes are often hidden by the complexity of the glycan profiles.

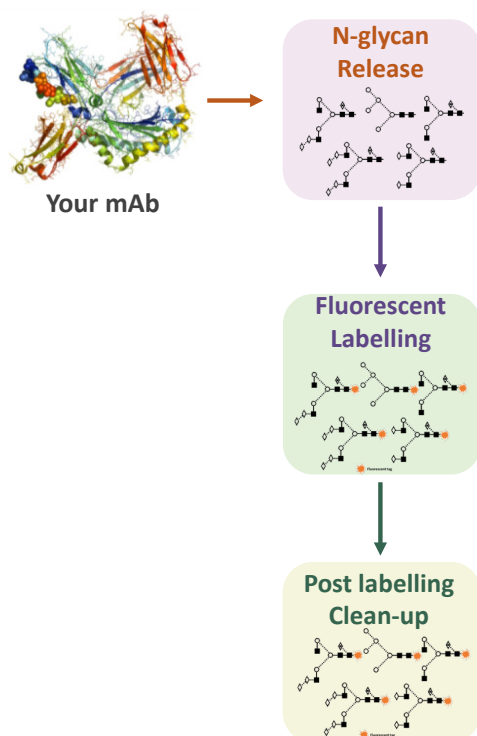
How Ludger can help?

1. Ludger's Technology

Ludger offers a range of products for analysis of biologics containing alpha-gal epitope. This includes: (a) kits for N-glycan release; (b) kits for glycan labelling; (c) glycan clean-up systems; (d) buffers for HILIC-UPLC systems; (e) System Suitability Controls; (f) Process Controls and Reference Standards.

Ludger's strategy that mAb developers can use for detection and quantification of Gal α 1-3Gal bearing glycans:

Ludger's Technology - for more info click on product code (or guide)



- N-glycan release kit: **LZ-rPNGaseF-kit (product guide)**
- Process Control to assess the release, labelling, clean-up and analyses: **GCP-IGG-100U** (100 μ g human IgG glycoprotein).
- Procainamide tag: **LT-KPROC-24 (product guide)**
- 2-AB Tag: **LT-KAB-A2 (product guide)**
- 2-AA Tag: **LT-KAA-A2 (product guide)**
- Process Control to assess the labelling, clean-up and analyses: **CLIBN-IGG-01** (N-glycans released from human IgG glycoprotein).
- Clean-up plate for procainamide labelled glycans: **LC-PROC-96 (product guide)**
- Clean-up cartridges for 2-AB or 2-AA labelled glycans: **LC-T1-A6 (product guide)**
- Buffer for HILIC-UPLC: **LS-N-BUFFX40 (product guide)**
- System Suitability Standard and Reference Standard for GU allocation: **CPROC-GHP-30 / CAB-GHP-30 / CAA-GHP-30** - Procainamide or 2-AB or 2-AA Labelled Glucose Homopolymer (GHP). *GU values can be used as a primary identification for glycans based on reported values in the literature and databases.*
- Reference Standards: Mixtures of N-glycans Common to mAb Samples: www.ludger.com/products/glycan-standards

- HILIC-FLR-UPLC
Or
- HILIC-FLR-UPLC-ESI-MS/MS (LC-MS/MS)

Exoglycosidase Digestions followed by:

- HILIC-FLR-UPLC
Or
- HILIC-FLR-UPLC-ESI-MS/MS (LC-MS/MS)

- Exoglycosidases:
 - Sialidase (removes α 2-3,6,8 sialic acids)
 - Fucosidase (removes α 1-2,3,4,6 fucose)
 - Beta Galactosidase (removes β 1-4 galactose)
 - Alpha Galactosidase (specific for α -galactose)
 - Beta-N-acetylglucosaminidase (specific for GlcNAcs β -linked to mannose)
 - Mannosidase (removes α 1-2,3,6 mannose)
www.ludger.com/products/exoglycosidases
- Post-exoglycosidase Cleanup Plate: **LC-PBM-96 (product guide)**
- Process Control to assess the exoglycosidase digestions and analyses: Mixtures of N-glycans Common to mAb Samples:
www.ludger.com/products/glycan-standards
- Process Control to assess the alpha galactosidase digestions and analyses: **CAB-ALPHA-GAL-01** Native or fluorescently labelled glycan containing the Gal α 1-3Gal

If you require any further information on how we can help you select products, please contact us at: info@ludger.com

2. Ludger's Glycan Analysis Services

Our strategy is to offer a range of complementary analytical methods (e.g. HILIC-UPLC, exoglycosidase sequencing, HILIC-FLR-ESI-MS/MS) to answer questions about the structures of glycans on glycoproteins. The complexity of the analysis reflects the detail of the information required. We work closely with clients **to design and execute appropriate glycoprofiling programmes** and can work up to GMP standard. Our data and **customised reports** are used: (i) in process optimisation; (ii) to support regulatory submissions; (iii) for lot release of drug batches during biomanufacturing.

We can also transfer and validate these optimised glycoprofiling methods to your laboratories.

Glycan Analysis Services



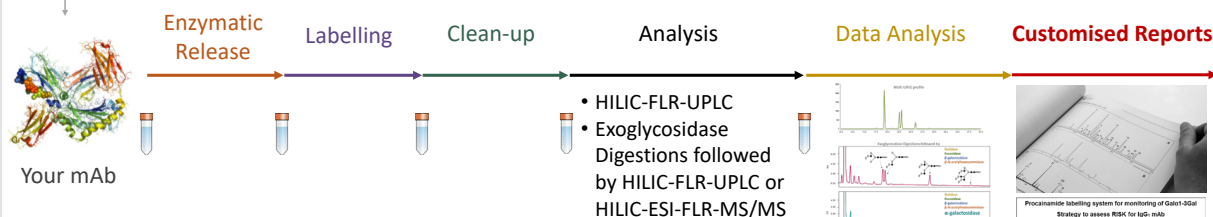
Dr Radoslaw Kozak
Head of Glycoprofiling
rad.kozak@ludger.com

- Discussion with Client regarding requirements
- CDA/MSA if required



Study Proposals & Quotation

- Client Purchase Order
- Client Samples



Ludger's reports are used by companies around the world:

- in process optimisation
- to support regulatory submissions
- for lot release of drug batches during biomanufacturing

We can transfer these methods to your laboratory

We can provide Compliance documentation for GMP

