




Ludger 2AB Labelling Kit - LT-KAB-A2

We would like to wish you all a healthy and prosperous 2020!

## How to optimize, measure and control Gal $\alpha$ 1-3Gal (non-human epitope) on your monoclonal antibody (mAb) therapeutics?

The clinical safety and efficacy of a glycoprotein drug is significantly influenced by its glycosylation. For instance, the presence of terminal  $\alpha$ 1-3-linked galactose (alpha-gal) can affect the safety profile and lead to a potential adverse reactions and neutralisation of the drug, thus reducing its therapeutic efficacy. Consequently, regulatory authorities have tightened the requirements for biopharmaceutical companies to characterise, control and monitor their therapeutics glycosylation. However, identification and quantitative analysis glycans can be difficult to achieve due to their complexity and heterogeneity.



## Alpha-Gal-containing biologics

Alfred W. Ludger, Cytogen Science Center, Oxfordshire, UK

Biologics containing the non-human antigen, *Sialyl (a)Gal*, can significantly decrease the clinical performance of therapeutic monoclonal antibodies (mAbs). The presence of *Sialyl (a)Gal* can affect safety profile and lead to a potential adverse reaction and neutralization of the drug by anti-*Sialyl (a)Gal* antibodies raised by patients using mAbs. *Sialyl (a)Gal* are a high priority Glycanomic Clinical Analyte (GCCA) and the development of assays must be iterative, complete, and ensure the identification of the mAb *Sialyl (a)Gal* levels throughout the product cycle.

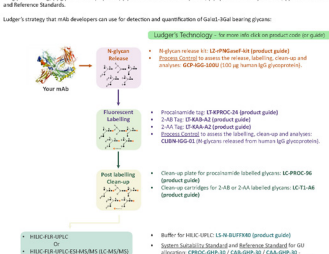
Detecting and quantifying the amount of *Sialyl (a)Gal* can be very difficult as these sugars are often hidden by the measure of the glycan profiles.

**How Ludger can help?**

**1. Ludger's Technology**

Using our range of products for analysis of biologics containing a *Sialyl (a)Gal* epitope. This includes (a) kits for the glycan release, (b) kits for glycan labeling, (c) glycan clean-up, (d) kits for HPLC-MS/MS, (e) glycan System Software, (f) Process Controls and Reference Standards.

Ludger's strategy that links developments on-site with detection and quantification of *Sialyl (a)Gal* bearing glycans:



**2. Ludger's Technology** - for more with data product and/or in gel:

- HPLC glycan release: 2-AB, 2-AP, 2-AB/2-AP (2-AB/2-AP is preferred)
- Glycan labeling: 2-AB, 2-AP, 2-AB/2-AP (2-AB/2-AP is preferred)
- Glycan clean-up: 2-AB, 2-AP, 2-AB/2-AP (2-AB/2-AP is preferred)
- Glycan analysis: 2-AB, 2-AP, 2-AB/2-AP (2-AB/2-AP is preferred)
- Glycan profiling: 2-AB, 2-AP, 2-AB/2-AP (2-AB/2-AP is preferred)
- Glycan reference standards: 2-AB, 2-AP, 2-AB/2-AP (2-AB/2-AP is preferred)

**3. Ludger's Technology** - for more with data product and/or in gel:

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- Glycan profiling: 2-AB, 2-AP, 2-AB/2-AP (2-AB/2-AP is preferred)
- Glycan reference standards: 2-AB, 2-AP, 2-AB/2-AP (2-AB/2-AP is preferred)

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- HPLC glycan release: 2-AB, 2-AP, 2-AB/2-AP (2-AB/2-AP is preferred)
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- Glycan clean-up: 2-AB, 2-AP, 2-AB/2-AP (2-AB/2-AP is preferred)
- Glycan analysis: 2-AB, 2-AP, 2-AB/2-AP (2-AB/2-AP is preferred)
- Glycan profiling: 2-AB, 2-AP, 2-AB/2-AP (2-AB/2-AP is preferred)
- Glycan reference standards: 2-AB, 2-AP, 2-AB/2-AP (2-AB/2-AP is preferred)

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- HPLC glycan release: 2-AB, 2-AP, 2-AB/2-AP (2-AB/2-AP is preferred)
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- Glycan clean-up: 2-AB, 2-AP, 2-AB/2-AP (2-AB/2-AP is preferred)
- Glycan analysis: 2-AB, 2-AP, 2-AB/2-AP (2-AB/2-AP is preferred)
- Glycan profiling: 2-AB, 2-AP, 2-AB/2-AP (2-AB/2-AP is preferred)
- Glycan reference standards: 2-AB, 2-AP, 2-AB/2-AP (2-AB/2-AP is preferred)

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- Glycan labeling: 2-AB, 2-AP, 2-AB/2-AP (2-AB/2-AP is preferred)
- Glycan clean-up: 2-AB, 2-AP, 2-AB/2-AP (2-AB/2-AP is preferred)
- Glycan analysis: 2-AB, 2-AP, 2-AB/2-AP (2-AB/2-AP is preferred)
- Glycan profiling: 2-AB, 2-AP, 2-AB/2-AP (2-AB/2-AP is preferred)
- Glycan reference standards: 2-AB, 2-AP, 2-AB/2-AP (2-AB/2-AP is preferred)

**7. Ludger's Technology** - for more with data product and/or in gel:

- HPLC glycan release: 2-AB, 2-AP, 2-AB/2-AP (2-AB/2-AP is preferred)
- Glycan labeling: 2-AB, 2-AP, 2-AB/2-AP (2-AB/2-AP is preferred)
- Glycan clean-up: 2-AB, 2-AP, 2-AB/2-AP (2-AB/2-AP is preferred)
- Glycan analysis: 2-AB, 2-AP, 2-AB/2-AP (2-AB/2-AP is preferred)
- Glycan profiling: 2-AB, 2-AP, 2-AB/2-AP (2-AB/2-AP is preferred)
- Glycan reference standards: 2-AB, 2-AP, 2-AB/2-AP (2-AB/2-AP is preferred)

**8. Ludger's Technology** - for more with data product and/or in gel:

- HPLC glycan release: 2-AB, 2-AP, 2-AB/2-AP (2-AB/2-AP is preferred)
- Glycan labeling: 2-AB, 2-AP, 2-AB/2-AP (2-AB/2-AP is preferred)
- Glycan clean-up: 2-AB, 2-AP, 2-AB/2-AP (2-AB/2-AP is preferred)
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- Glycan profiling: 2-AB, 2-AP, 2-AB/2-AP (2-AB/2-AP is preferred)
- Glycan reference standards: 2-AB, 2-AP, 2-AB/2-AP (2-AB/2-AP is preferred)

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- HPLC glycan release: 2-AB, 2-AP, 2-AB/2-AP (2-AB/2-AP is preferred)
- Glycan labeling: 2-AB, 2-AP, 2-AB/2-AP (2-AB/2-AP is preferred)
- Glycan clean-up: 2-AB, 2-AP, 2-AB/2-AP (2-AB/2-AP is preferred)
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- Glycan reference standards: 2-AB, 2-AP, 2-AB/2-AP (2-AB/2-AP is preferred)

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- HPLC glycan release: 2-AB, 2-AP, 2-AB/2-AP (2-AB/2-AP is preferred)
- Glycan labeling: 2-AB, 2-AP, 2-AB/2-AP (2-AB/2-AP is preferred)
- Glycan clean-up: 2-AB, 2-AP, 2-AB/2-AP (2-AB/2-AP is preferred)
- Glycan analysis: 2-AB, 2-AP, 2-AB/2-AP (2-AB/2-AP is preferred)
- Glycan profiling: 2-AB, 2-AP, 2-AB/2-AP (2-AB/2-AP is preferred)
- Glycan reference standards: 2-AB, 2-AP, 2-AB/2-AP (2-AB/2-AP is preferred)

**11. Ludger's Technology** - for more with data product and/or in gel:

- HPLC glycan release: 2-AB, 2-AP, 2-AB/2-AP (2-AB/2-AP is preferred)
- Glycan labeling: 2-AB, 2-AP, 2-AB/2-AP (2-AB/2-AP is preferred)
- Glycan clean-up: 2-AB, 2-AP, 2-AB/2-AP (2-AB/2-AP is preferred)
- Glycan analysis: 2-AB, 2-AP, 2-AB/2-AP (2-AB/2-AP is preferred)
- Glycan profiling: 2-AB, 2-AP, 2-AB/2-AP (2-AB/2-AP is preferred)
- Glycan reference standards: 2-AB, 2-AP, 2-AB/2-AP (2-AB/2-AP is preferred)

**12. Ludger's Technology** - for more with data product and/or in gel:

- HPLC glycan release: 2-AB, 2-AP, 2-AB/2-AP (2-AB/2-AP is preferred)
- Glycan labeling: 2-AB, 2-AP, 2-AB/2-AP (2-AB/2-AP is preferred)
- Glycan clean-up: 2-AB, 2-AP, 2-AB/2-AP (2-AB/2-AP is preferred)
- Glycan analysis: 2-AB, 2-AP, 2-AB/2-AP (2-AB/2-AP is preferred)
- Glycan profiling: 2-AB, 2-AP, 2-AB/2-AP (2-AB/2-AP is preferred)
- Glycan reference standards: 2-AB, 2-AP, 2-AB/2-AP (2-AB/2-AP is preferred)

**13. Ludger's Technology** - for more with data product and/or in gel:

- HPLC glycan release: 2-AB, 2-AP, 2-AB/2-AP (2-AB/2-AP is preferred)
- Glycan labeling: 2-AB, 2-AP, 2-AB/2-AP (2-AB/2-AP is preferred)
- Glycan clean-up: 2-AB, 2-AP, 2-AB/2-AP (2-AB/2-AP is preferred)
- Glycan analysis: 2-AB, 2-AP, 2-AB/2-AP (2-AB/2-AP is preferred)
- Glycan profiling: 2-AB, 2-AP, 2-AB/2-AP (2-AB/2-AP is preferred)
- Glycan reference standards: 2-AB, 2-AP, 2-AB/2-AP (2-AB/2-AP is preferred)

**14. Ludger's Technology** - for more with data product and/or in gel:

- HPLC glycan release: 2-AB, 2-AP, 2-AB/2-AP (2-AB/2-AP is preferred)
- Glycan labeling: 2-AB, 2-AP, 2-AB/2-AP (2-AB/2-AP is preferred)
- Glycan clean-up: 2-AB, 2-AP, 2-AB/2-AP (2-AB/2-AP is preferred)
- Glycan analysis: 2-AB, 2-AP, 2-AB/2-AP (2-AB/2-AP is preferred)
- Glycan profiling: 2-AB, 2

Our new application note illustrates Ludger's expertise and the range of glycan analysis services for detection of alpha-gal and how we can assist you with identifying the Glycosylation Critical Quality Attributes (GCQA's) of your glycoprotein drug.

We offer alpha-Gal standards labelled with 2-AB [CAB-Alpha-Gal-01] and 2-AA [CAA-AlphaGal-01]. These standards can be used as positive controls in glycoprofiling sequencing experiments utilising alpha 1-3 galactose specific exoglycosidase.


To enquire regarding glycan analysis please contact [rad.kozak@ludger.com](mailto:rad.kozak@ludger.com) or to place an order please contact [info@ludger.com](mailto:info@ludger.com)

For more information and to view the application note, please visit our [webpage on glycan analysis](#).

## Glycan Derivatization

Most methods for protein glycosylation analysis rely on the release of glycans from the glycoprotein. Released glycans lack chromophore or fluorophore properties which restrict their detection and separation by liquid chromatography (LC), mass spectrometry (MS) or a combination of both.

Ludger addresses these challenges by providing several tags which enable glycan detection and separation by LC and enhance ionization efficiency during MS analysis.

 LudgerTag Labelling Kits	LT-KAB-A2	LT-KAB-VP24	LT-KAB-VP96	LT-KAA-A2	LT-KAA-VP24	LT-KPROC-24	LT-KPROC-VP24	LT-KPROC-96	LT-KDMB-A1	LT-VTAG-24	LT-VTAG-C30	LT-MONO-96	LT-PERMET-96**	LT-PERMET-VP96**
Application:														
N-glycans	✓	✓	✓	✓	✓	✓	✓	✓			✓		✓	✓
O-glycans	✓	✓	✓	✓	✓	✓	✓	✓					✓	✓
GSL glycans	✓	✓	✓	✓	✓	✓		✓					✓	✓
IgG glycopeptides										✓				
Sialic acids									✓					
Monosaccharides												✓		
Release*									included		included	included		
Label	2AB	2AB	2AB	2AA	2AA	Procainamide	Procainamide	Procainamide	DMB	V-Tag	V-Tag	2AA	Permethylation	Permethylation
Reductant:														
Sodium Cyanoborohydride	✓			✓		✓		✓				✓		
Picolone borane		✓	✓		✓		✓							
Analytical platform:														
HPLC analysis	• • •	• • •	• • •	• • •	• • •	• • •	• • •	• • •	• • •	• • •	• • •	• • •		
UHPLC analysis	• • •	• • •	• • •	• • •	• • •	• • •	• • •	• • •	• • •	• • •	• • •	• • •		
LC-ESI-MS analysis	• •	• •	• •	• •	• •	• • •	• • •	• • •			•		• •	• •
MALDI-MS		• •	• •	• •	• •	• •				• •			• • •	• • •
Number of samples	24	24	96	24	24	24	24	96	22	24	30	96	96	96

Analytical platform sensitivity range: ●● (medium) — ●●● (high)  
MS signal detection can be affected with sample purity and presence of salt contaminants

\* for release of N-glycans use PNGase F (Cat# E-PNG-xx or LZ-rPNGase-F-kit), for O glycans use Ludger/Liberate Orela kit (Cat# LL-ORELA-A2) or hydrazinolysis kit (LL-HYDRAZ-A2), for GSLs use ceramide glycanase (Cat# LZ-CER-1IM-KIT), for IgG glycopeptides use protease

enzyme e.g. trypsin.

\*\*\* without Methyl iodide. This kit can be shipped outside of the UK.

The table above summarizes the applications, type of label, reductant method and analytical platform for each LudgerTag kit.

To view visit our [webpage](#) featuring our labelling technology.

