

Procainamide Glycan Labelling

- Features and benefits

Sensitive glycan analysis using
(U)HPLC, ESI-MS, and LC-ESI-MS



Features and benefits of procainamide labelling of glycans

1. Enhanced MS ionisation and fluorescence in comparison to 2-AB

Makes it applicable for UHPLC , ESI-MS, and LC-ESI-MS/MS analysis. Equivalent workflow and labelling chemistry as 2-AB

2. Suitable for analysis of N-, O-, and glycosphingolipid (GSL) glycans

From biopharmaceutical and biological samples

3. Procainamide labelled glycan standards

Range of system suitability standards available

4. Suitable for small sample size of 25pmol – 25nmol

Allows for identification of low abundant glycan species

5. Validated according to ICHQ2(R1) industry standard

Suitable for GMP work



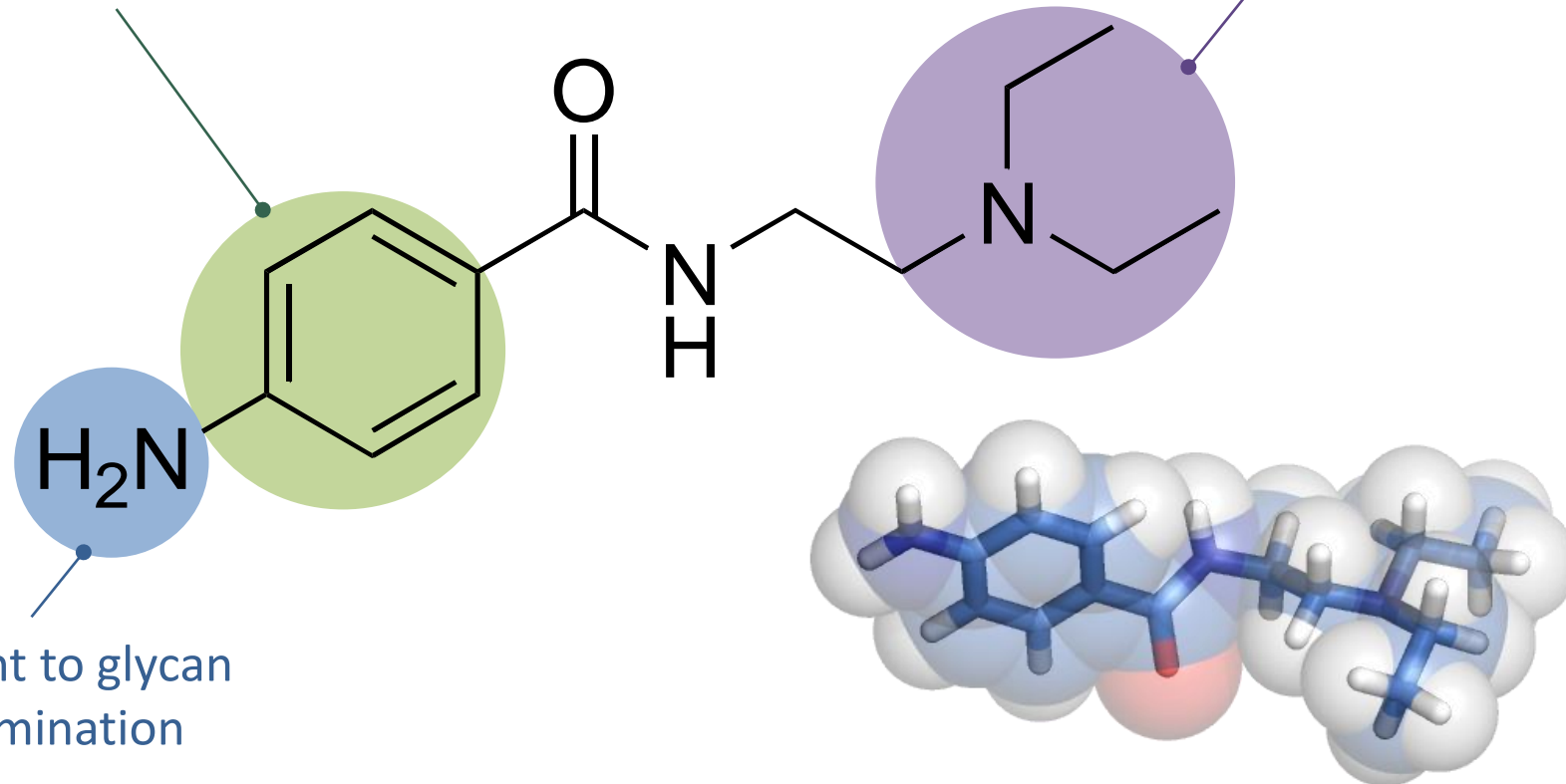
The anatomy of procainamide

4-amino-N-(2-diethylaminoethyl) benzamide

2-(diethylamino)ethyl group enhances the ionization efficiency in the positive ESI mode

Fluorescent group for detection at Ex 310 and Em 370 nm in UHPLC

Added mass
219.172 Da



Site of attachment to glycan
via reductive amination

LudgerTag Procainamide Labelling Kits

Our procainamide labelling technology uses the same *reductive amination* labelling method that has been used for 2AB & 2AA. The following procainamide labelling kits are available:

Kit name	Reductant	Kit size
LT-KPROC-24	Sodium cyanoborohydride	24 samples
LT-KPROC-96		96 samples
LT-KPROC-VP24	2-picoline borane	24 samples

Sodium cyanoborohydride is a gold standard reductant used in glycan labelling. Best practice is to perform the labelling in a fume cupboard.

2-picoline borane (2-PB) is less toxic than sodium cyanoborohydride and can be used 'on a laboratory bench'.



Workflow - LudgerTag Procinamide for glycan profiling and identification



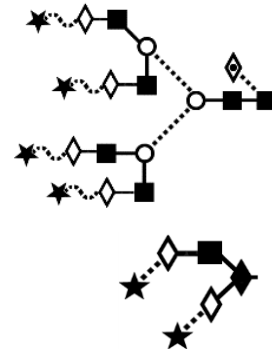
**Glycoprotein
or GSL**

**Enzymatic or
Chemical
Release**

15 min to 24 h
Depending on release
method and sample

Clean-up

1h

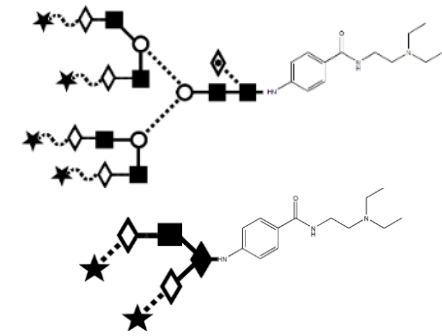


Labelling

1h

Clean-up

1h



**UHPLC
ESI-MS
LC-ESI-MS/MS**

Recommended Products

N-glycans	PNGase F (LZ-rPNGaseF-kit)	*Protein Binding Membrane (PBM) plate (LC-PBM-96)	Procainamide (LT-KPROC-24 LT-KPROC-96 LT-KPROC-VP24)	Procainamide plate (LC-PROC-96) or S-cartridges (LC-S-A6)
O-glycans	Hydrazine (LL-HYDRAZ-A2) or Orela (LL-ORELA-A2)	Cation Exchange (CEX) cartridges (LC-CEX-A6)		S-cartridges (LC-S-A6)
GSL	Ceramidase (LZ-CER-HM-KIT)	*Protein Binding Membrane (PBM) plate (LC-PBM-96)		Procainamide plate (LC-PROC-96) or S-cartridges (LC-S-A6)

*optional

Standards for procainamide workflow



Process controls:

Glycoproteins – IgG, Fetuin

Unlabelled glycans – including:

- N- and O-glycans
- Purified di-, tri-, tetraantennary glycans
- High mannose glycans
- Glycan libraries

System suitability standards:

Labelled glycans:

- Purified di-, tri-, tetraantennary glycans
- High mannose glycans
- Glycan libraries

Labelled Glucose Homopolymer (GHP)

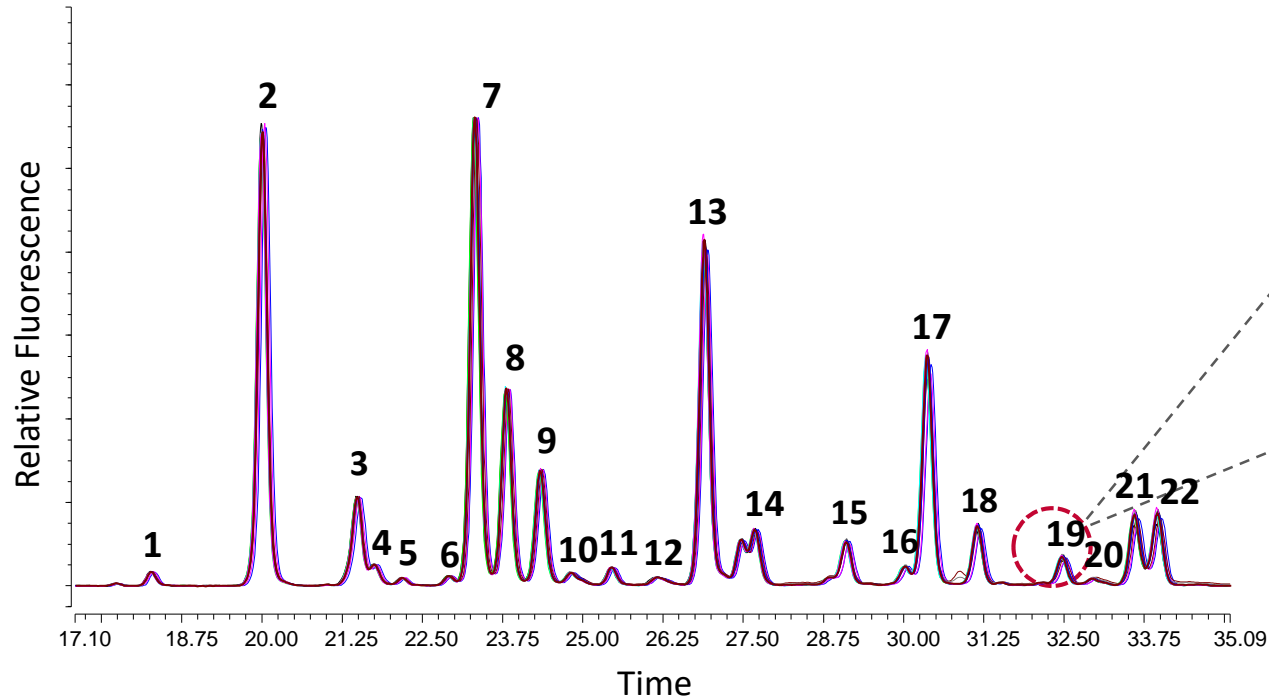
Ludger System Suitability Standards and Controls		Process controls (check for correct labeling)		Sample Protein (check for correct)		Labeling Process (check for correct)		MS System Suitability		Ludger System Suitability		MS System Suitability		System Identification by accurate mass or MS/MS matching		Quantification		End-user control		
Product Category	Ludger Code	Product Description	MS	MS/MS	MS	MS/MS	MS	MS/MS	MS	MS/MS	MS	MS/MS	MS	MS/MS	MS	MS/MS	MS	MS/MS	MS	MS/MS
Glycoproteins/Glycosylated	IGP-IGG	IgG Glycoprotein	+	+	+	+	+	+												
	IGP-FET	Fetuin Glycoprotein	+	+	+	+	+													
	IGP-IPR-2022	IGP-IPR-2022	+	+	+	+	+													
Nucleoside and Sulfic Acid standards	CM-SP	SP Sulfic Acid Reference Panel																		
	CM-NEU-AC	Neu5Ac																		
	CM-NEU-6C	Neu6C																		
	CM-NEU-6AC2	Neu6Ac																		
	CM-MON-6AC	Mon6Ac																		
Unlabelled N-Glycans	CM-XYL	Xylose																		
	CM-x	BL, T1 and Tera antennary N-glycans																		
	CM-Man-x	High Mannose N-glycans																		
	BO-CHITROSE	Chitinose																		
	BO-CH-MANN	Mann																		
	CLIM-IGG	IgG N-glycan library																		
	SL-MAN-x	Man, a glycan ref panel																		
2-AB labelled glycans	CAB-GHP	2-AB labelled Glucose Homopolymer																		
	BO-CAB-CH	2-AB labelled Chitinose																		
	CAB-IGG	2-AB labelled IgG N-glycan library																		
2-AA labelled glycans	CAB-x	BL, T1 and Tera antennary N-glycans																		
	CAB-Man-x	High Mannose N-glycans																		
	CAB-Glc-x	Glycans																		
	CAB-AlphaGal	Alpha-Gal standard																		
APTS labelled glycans	CAA-GHP	2-AA labelled Glucose Homopolymer																		
	BO-CAA-CH	2-AA labelled Chitinose																		
	CAA-x	BL, T1 and Tera antennary N-glycans																		
	CAA-Man-x	High Mannose N-glycans																		
PROC labelled glycans	CAPT-x	APTS labelled IgG N-glycan library																		
	CAPT-x	APTS labelled N-glycans																		
Permethyated Glycan Standards	CPM-IGG	Permethyated IgG N-glycans																		
	CPM-CL-IGG	Permethyated 13C IgG N-glycans																		

For full list of standards check comparison table:

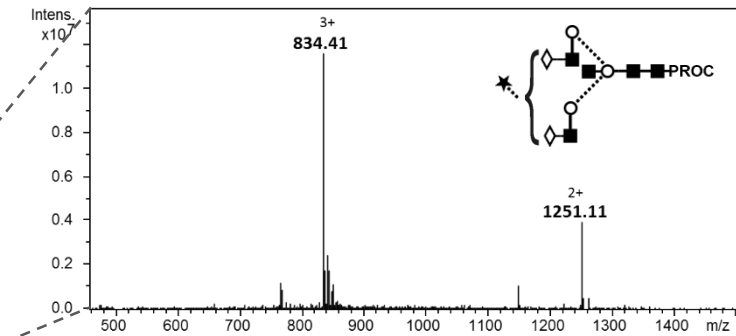
<https://www.ludger.com/system-suitability-standards>

Procainamide LC-MS analysis of human IgG

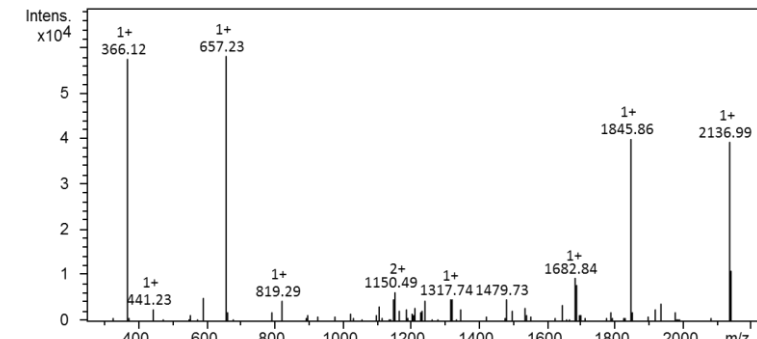
One system for relative quantitation (HILIC-UHPLC) and identification of glycans (ESI-MS)



*HILIC-UHPLC chromatogram of procainamide labelled human IgG
13 replicates overlaid*



ESI-MS data for FA2BG2S1 glycan



MS/MS data for FA2BG2S1 glycan

For more information and example applications of procainamide view this presentation:

<https://www.ludger.com/presentation/ludger-procainamide-glycan-labeling.pdf>

How to start using the Procainamide Glycan Labelling System

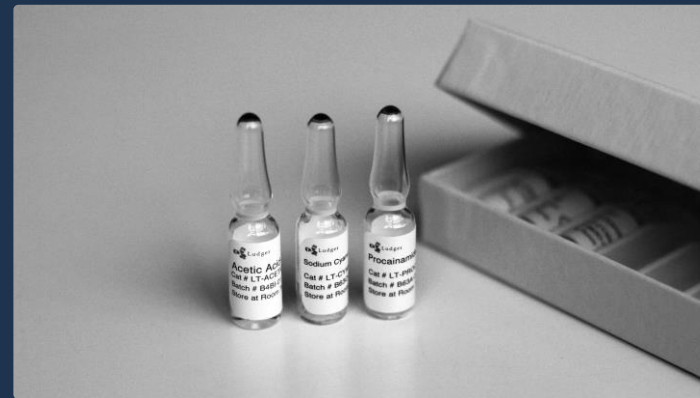
If you have a question



CLICK
to contact
Rad

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

Request a quotation



CLICK
to contact
Sales

Sales Team
info@ludger.com