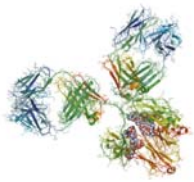

Product Guide for LudgerSep™ C2 anion exchange HPLC Columns for Glycan Analysis

(Ludger Product Codes: LS-C2-4.6x50 and LS-C2-4.6x150)

Ludger Document # LS-C2-Guide-v3.0

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Specifications for LudgerSep™ C2 Columns

Application

Charged-based analysis and purification by HPLC of LudgerTag™ fluorophore and UV-chromophore labeled glycans.

Description

LudgerSep™C2 HPLC columns contain particles with a macroporous polymeric anion exchange coating optimized for anion exchange chromatography of complex glycan mixtures.

Particles

8 μm polystyrene divinyl benzene with a stable quaternized polyethylene imine coating

Cat #	Diameter x Length	Column Volume
LS-C2-4.6x50	4.6 x 50 mm	0.83 ml
LS-C2-4.6x150	4.6 x 150 mm	2.49 ml

Column Tube

Stainless steel

Flow Rates

Typical flow rates = 0.3 – 2.0 ml/min.
Maximum flow rate = 4 ml/min

Pressure

Pressure should not exceed 300psi (207bar)

pH Range

1 – 13

Temperature

Typical operating temperature = 30 ^{o}C, but increasing the temperature may improve resolution for some samples.
Maximum temperature range = 10 - 60 ^{o}C.

Solvents

Typical solvent systems for glycan analysis include gradients of aqueous buffers containing acetonitrile and either ammonium formate , (cat. No. LS-N-BUFFX40) ammonium acetate, or sodium acetate salts.
Avoid strong oxidants and anionic detergents.

Column Protection

Filter all solvents to 0.2 μm and degas using either helium sparging or vacuum degassing.
Filter all samples using a 0.2 μm filter membrane before loading onto the column.
Install a good quality in-line filter between the sample injector and the column.
Please call us for advice on the most suitable sample and in-line filters to use.

Amount of Sample The maximum amount of glycan sample that can be loaded on the column depends on the number and type of glycan components as well as the nature of any non-glycan material. The typical range for successful analytical runs is 1pmol - 1 nmol per sample peak and up to 200 nmol of total glycans.

Suitable Samples Suitable samples include glycans labelled with the following LudgerTag labels :
2-AA (2-aminobenzoic acid),
2-AB (2-aminobenzamide exchange)

Sample Preparation Filter samples to 0.2 μm then dry using a centrifugal evaporator.
Re-dissolve in 5 - 50 μl of the starting buffer (i.e. the solvent mixture used at the very start of the HPLC gradient) then inject.

Sample Detection Either fluorescence or UV-absorbance depending on the dye used (see the appropriate LudgerTag instruction guide).

Handling: Ensure that any glass, plasticware or solvents used are free of glycosidases and environmental carbohydrates. Use powder-free gloves for all sample handling procedures and avoid contamination with environmental carbohydrate.

Safety: Please read the Material Safety Data Sheets (MSDS's) for all chemicals used.
All processes involving labelling reagents should be performed using appropriate personal safety protection - eyeglasses, chemically resistant gloves (e.g. nitrile), etc. - and where appropriate in a laboratory fume cupboard

For research use only. Not for human or drug use

HPLC System Requirements

LudgerSep™ C2 columns can be used with any HPLC pumping system capable of delivering accurate gradients at a flow rate of 0.3 to 2.0 ml/min. In general, systems which mix eluants at high pressure (after the pump head) have lower dead volumes and supply more accurate gradients that are appropriate at the flow rate needed for LudgerSep columns. Low dead volume injectors should be used (Rheodyne 7125 / 9125 or similar are recommended). The loop size to be used depends on the separation mode and amount of sample. For analytical runs it is desirable to minimise the sample volume and, typically, a 10 μl loop is used with sample injection volumes of 1 to 5 μl (partial fill) or > 10 μl (complete fill). For charge mode separations, generally, anionic glycans which are retained by the column (and are therefore effectively concentrated on the

column) are reasonably tolerant of larger injection volumes whereas non-anionic glycans are not retained by the column matrix and will elute in a volume proportional to the injection volume.

A fluorescence detector is required (λ_{ex} = 330 nm, λ_{em}=420 nm) for LudgerTag 2-AB or 2-AA labelled oligosaccharides. This could be either a filter or monochromator type. Fluorescence filters for OPA-peptide analysis work well and, typically, with these glycan peaks eluting from the LudgerSep C2 column containing 2 - 5 pmol of 2-AB or 2-AA labelled glycans can be detected with good signal-to-noise.

LudgerTag™ 2-AB and 2-AA labelled glycans can also be detected using UV (254 nm) with an approximately 10 to 100 fold reduction in sensitivity compared to fluorescence. Unlabeled oligosaccharides, particularly those bearing N-acyl amino groups, can be detected at 190 - 210 nm. down to the 1 nmol level. However, the absorption of the buffers and solvents becomes a problem when using UV detection, especially at short wavelengths.

Installation of the Column

During column installation we recommend that :

- You should connect the LudgerSep C2 column to your HPLC system using standard 1/16” OD tubing and Valco compatible fittings in either stainless steel or PEEK (polyetheretherketone). Hand-tight PEEK fittings and tubing (0.17 mm / 0.007” ID) are recommended for ease of connection and to minimise damage to the column threads. Flow direction is indicated by an arrow on the column label.
- Keep the lengths of tubing between the injector to column and column to detector as short as possible to minimise dispersion effects.
- Install an in-line filter with minimal dead volume either immediately before the injector or between the injector and the head of the LudgerSep C2 column to prevent damage to the column by particles.
- Before analysing any samples, the newly installed column should be conditioned using the protocol described below.

Preconditioning of the Newly Installed Column

The following preconditioning steps are recommended prior to use of the column :

Flush the column sequentially at a flow rate of 1.0 ml/min with the following eluants in order :

- Low ionic strength component of buffer A, for example 0.01M Tris HCl, pH 8.0, for 5 minutes
- High ionic strength component of buffer B, for example 0.01 M Tris HCl, 0.5 M NaCl, pH 8.0. Continue with this eluent until a stable baseline is achieved at the required sensitivity.
- Equilibrate with buffer A for 5 minutes.
- Equilibrate with starting buffer.

Column Cleaning and Storage

After heavy use, your LudgerSep C2 column may become contaminated with strongly adsorbed sample constituents that will lead to a loss in column performance.

- If the contamination is light, clean the column following the protocol in the 'Column Preconditioning' section above. Afterwards, equilibrate in the appropriate starting buffer and run a charge mode gradient without injecting a sample to check the baseline.
- If more rigorous cleaning is required, wash with 0.1 M acid, e.g. acetic or hydrochloric and 0.1M base e.g. sodium hydroxide.
- If the contamination is due to small hydrophobic molecules e.g. fats, detergents and peptides, then the matrix should be washed with an organic alcohol e.g. isopropanol.
- The addition of 0.1% trifluoroacetic acid may be advantageous.
- Take the column through two complete LudgerSep C2 charge mode cycles without any sample injected.
- Column cleaning is most effective at a low flow rate (e.g. 0.2 ml / min).
- For long-term storage, the column should be washed with 1M solution chloride.
- After flushing with water the storage buffer of approximately 0.1 M Na_2SO_4 containing 0.2% sodium azide can be introduced. Keep at room temperature.

Sample Preparation

Samples intended for charge mode analysis on LudgerSep C2 columns must be free of salt or anionic detergent and free of any particulates.

Anionic glycans cannot be desalted using a simple mixed-bed ion exchange resin since they will themselves be retained by the resin. Volatile salts can be removed by repetitive drying in a centrifugal evaporator using a high vacuum followed by dissolution in water (typically 50 - 100 µl) until there is no visible salt residue.

Alternatively, desalting can be achieved using a LudgerClean EB10 cartridge. If a LudgerClean EB10 cartridge is used then make sure that all of the trifluoroacetic acid (used as a trace additive for elution from the cartridge) is removed from the sample by repetitive drying and dissolution as described above. This is to ensure that any sialylated glycans are fully de-protonated prior to injection onto the column (see Appendix 1, section A.2).

Sialylated glycans can become desialylated if exposed to acidic conditions and elevated temperatures. Avoid desialylation with such samples by (a) minimising exposure to acid (if possible, keep the pH above 5), and (b) minimising exposure to temperatures greater than 25^{o}C.

Particulates can be removed from samples using microcentrifuge filters with 0.2 μm pore size membranes.

Charge Mode Analysis of Anionic and Neutral Glycans

Picomole quantities of oligosaccharides may be analysed by fluorescent labelling with 2-aminobenzamide (2-AB) using the LudgerTag 2-AB Glycan Labelling Kit (Cat. No. LT-KAB-A2) or with 2-aminobenzoic (2-AA) using the LudgerTag 2-AA Glycan Labelling Kit (Cat # LT-KAA-A2), followed by LudgerSep C2 HPLC.

The outline of the procedure is as follows :

- The oligosaccharides are labelled by reductive amination with 2-AB or 2-AA.
- Excess labelling reagents are then removed using LudgerClean™ S cartridges.
- The labelled oligosaccharides are analysed by anion exchange HPLC on a LudgerSep™ C2 column with fluorescence detection.

The following protocols are intended as a guide to the conditions for using LudgerSep™ C2 columns. Always use HPLC grade buffer salts and solvents together with pure water. It is recommended that buffers are prepared from the appropriate acid and base, e.g. formic acid and ammonium hydroxide, as these are generally available in a purer grade than the corresponding salts. Buffer concentrations are always expressed in terms of the anion. Always titrate an accurately measured amount of acetic acid or formic acid (aq) with ammonium hydroxide to the desired pH value. Only mix separately measured volumes of acetonitrile with water or aqueous buffers - do not ‘top-up’ aqueous solutions with organic solvents (or vice versa). Lastly, it is important to thoroughly degas buffers before use. This can be achieved using sonication, sparging with helium, or vacuum degassing.

Column: LudgerSep C2 - 4.6 x 150 mm

Solvents:	Solvent A = water		Solvent B = 200 mM sodium acetate	
Flow rate:	1 ml/min.			
Gradient :	time / min	% A	% B	Comments
	0	100	0	
	1	100	0	
	20	60	40	
	22	60	40	
	25	100	0	
	39	100	0	
	40	100	0	change flow rate to 0.05 ml/min

Note: Higher percentage solvent B may be required for glycans with three or more negative charges.

Detection by fluorescence :

λ_{ex} = 330 nm, λ_{em} =420 nm

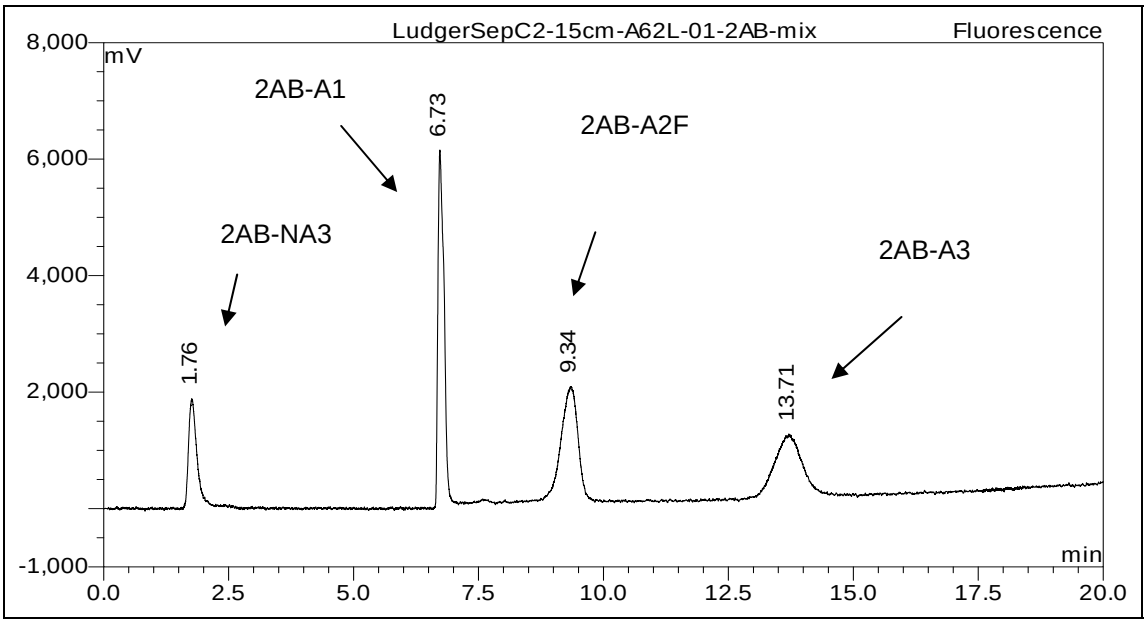

Figure 1: Separation of 2AB labelled glycan standards (picomolar concentration) LudgerSepC2 4.6 x 150 mm.

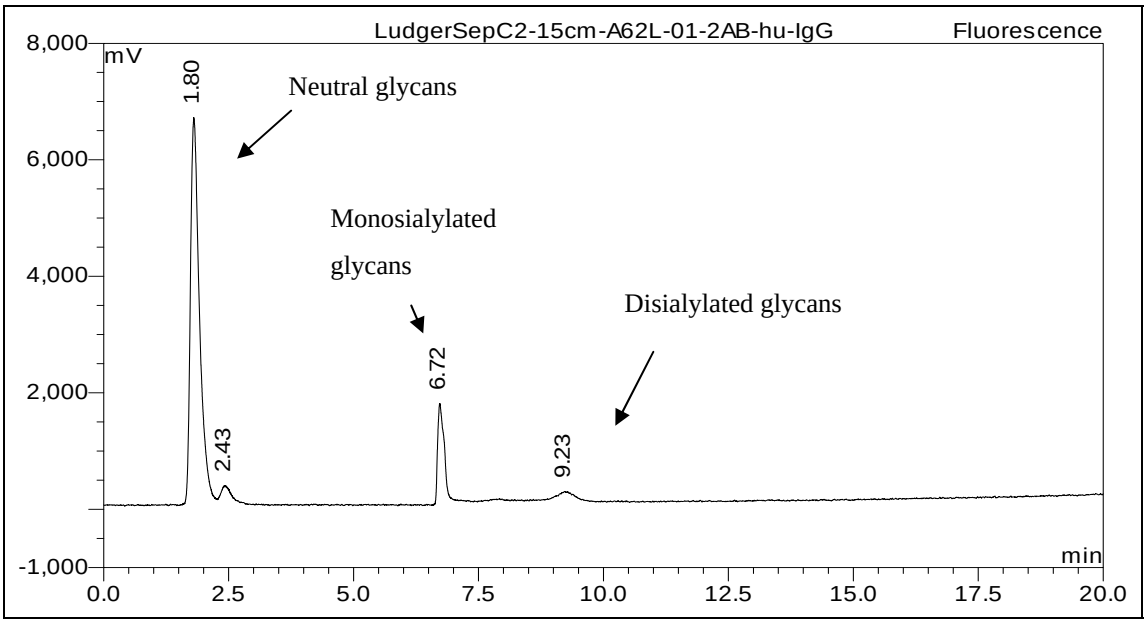

Figure 2: Separation of 2AB human IgG glycans (Cat. No. CAB-HUIGG-01). LudgerSepC2 - 4.6 x 150 mm.

Warranties and Liabilities

Ludger warrants that the above product conforms to the attached analytical documents. Should the product fail for reasons other than through misuse Ludger will, at its option, replace free of charge or refund the purchase price. This warranty is exclusive and Ludger makes no other warrants, expressed or implied, including any implied conditions or warranties of merchantability or fitness for any particular purpose.
Ludger shall not be liable for any incidental, consequential or contingent damages.

This product is intended for *in vitro* research only.

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