



# **LudgerSep<sup>™</sup> C3 Anion Exchange HPLC Column for Glycan Analysis**

## **Product Guide**

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## LudgerSep C3 Glycan Analysis HPLC Column - Specifications

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<b>Application</b>	Charged-based analysis and purification by HPLC of LudgerTag™ fluorophore and UV-chromophore labeled glycans.	
<b>Description</b>	The C3 HPLC column contains macroporous (1000 Angstrom) anion exchange particles optimized for anion exchange chromatography of complex glycan mixtures.	
<b>Particles</b>	10 µm polymer based hydrophilic particles. Functional group $-\text{CH}_2\text{CH}_2\text{N}^+(\text{CH}_2\text{H}_5)_2$	
<b>Column Size</b>	<b>Cat #</b>	<b>Diameter x Length</b>
	LS-C3-7.5x75	7.5 x 75 mm
<b>Column Tube</b>	Stainless steel	
<b>Flow Rates</b>	Typical flow rates = 0.3 – 1.0 ml/min Maximum flow rate = 1.2 ml/min	
<b>Pressure</b>	Pressure should not exceed 150psi	
<b>pH Range</b>	2 – 12	
<b>Temperature</b>	Typical operating temperature = 30 °C, Maximum temperature range = 10 - 45 °C.	
<b>Solvents</b>	Shipping Solvent – deionized water. Typical solvent systems for glycan analysis include gradients of aqueous buffers containing acetonitrile and either ammonium formate, ammonium acetate, or sodium acetate salts (maximum 20% acetonitrile and 0.5 Molar salt).	
<b>Column Protection</b>	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 an in-line 0.2 µm filter with minimal dead volume between the injector and the head of the column to prevent damage to the column by particles.	

- 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 0.1 pmol - 1 nmol per sample peak and up to 200 nmol of total glycans.
- Suitable Samples** Suitable samples include glycans labeled with the following LudgerTag labels :  
2-AB (2-aminobenzamide)
- Sample** Filter samples to 0.2 µm then dry using a centrifugal evaporator.
- Preparation** Re-dissolve in 5 - 50 µl of water then inject.
- Sample Detection** Fluorescence detector.
- 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 labeling reagents should be performed using appropriate personal safety protection – safety glasses, 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**

## Installation

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During column installation we recommend that :

- You should connect the LudgerSep C3 column to the HPLC using PEEK fittings and tubing (10.32 (1/16") ID). Hand-tightened fittings 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 0.2 µm in-line filter with minimal dead volume either immediately before the injector or between the injector and the head of the LudgerSep C3 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.

## Column Preconditioning

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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 :

- Solvent A - 20% acetonitrile for 30 minutes.
- Solvent B - 500 mM ammonium acetate pH 4.5 in 20% acetonitrile, or 500 mM ammonium formate pH 4.4 in 20 % acetonitrile for 30 minutes.
- Equilibrate with Solvent A for 30 minutes.

## Column Cleaning and Storage

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After heavy use, your LudgerSep C3 column may become contaminated with strongly adsorbed sample constituents that will lead to a loss in column performance.

- Clean the column following the protocol in the 'Column Preconditioning' section above. Afterwards, equilibrate in the appropriate starting buffer and run a gradient without injecting a sample to check the baseline.
- Store the column in Solvent A – 20% acetonitrile.

## Sample Preparation

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Samples intended for charge mode analysis on LudgerSep C3 columns must be free of salt or anionic detergent and free of any particulates.

Sialylated glycans can become desialylated if exposed to acidic conditions with elevated temperatures. Avoid desialylation with such samples by (a) minimising long-term storage exposure to acidic conditions (if possible, keep the pH above 5), and (b) minimising exposure to temperatures greater than 25°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

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Picomole quantities (or less depending on the sensitivity of the detector) of oligosaccharides may be analysed by fluorescent labeling with 2-aminobenzamide (2-AB) using the LudgerTag 2-AB Glycan Labeling Kit (Cat. No. LT-KAB-A2) followed by LudgerSep C3 HPLC.

The outline of the procedure is as follows :

- The oligosaccharides are labeled by reductive amination with 2-AB.
- Excess labeling reagents are then removed using LudgerClean S cartridges.
- The labeled oligosaccharides are analysed by anion exchange HPLC on a LudgerSep™ C3 column with fluorescence detection.

The following protocols are intended as a guide to the conditions for using LudgerSep C3 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 (to produce ammonium formate), or acetic acid with ammonium hydroxide (to produce ammonium acetate) 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. Lastly, it is important to thoroughly degas buffers before use. This can be achieved using sonication, sparging with helium, or vacuum degassing.

Below are the details of two types of solvent system used at Ludger. The ammonium formate system is particularly useful if samples are to be collected after elution from the column and run on the LudgerSepN1/N2 columns for orthogonal separation.

## Ammonium Acetate Solvent

**Column:** LudgerSep C3 – 7.5 x 75 mm

**Solvents:** Solvent A = 20% acetonitrile.

Solvent B = 500 mM ammonium acetate pH 4.5 in 20% acetonitrile.

**Flow rate:** 0.8 ml/min.

Gradient :	time / min	% A	% B	Comments
	0	100	0	
	5	100	0	
	21	96	4	
	61	75	25	
	72	60	40	
	75	60	40	
	76	100	0	
	90	100	0	

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

### Detection by fluorescence :

$\lambda_{ex} = 330 \text{ nm}$ ,  $\lambda_{em} = 420 \text{ nm}$

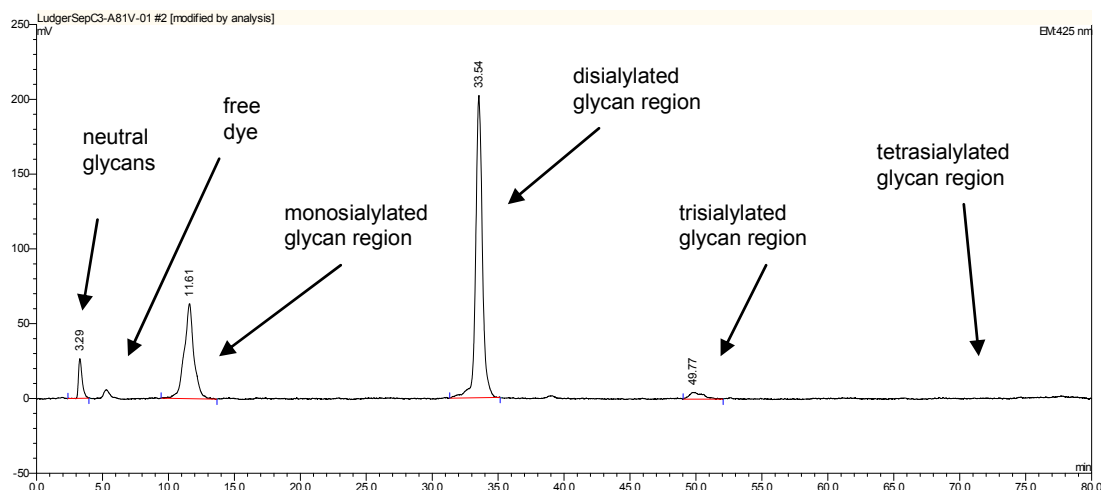


Figure 1: Separation of 2AB labeled glycan standards (picomolar concentration) on LudgerSepC3 7.5 x 75mm using ammonium acetate based solvent.

## Ammonium Formate Solvent

**Column:** LudgerSep C3 – 7.5 x 75 mm

**Solvents:** Solvent A = 20% acetonitrile.

Solvent B = 500 mM ammonium acetate pH 4.5 in 20% acetonitrile.

**Flow rate:** 0.4-0.8 ml/min.

Gradient :	time / min	% A	% B	Flow Rate ml/min
	0	100	0	0.4
	5	100	0	0.4
	21	96	4	0.4
	61	75	25	0.4
	72	60	40	0.4
	75	60	40	0.4
	76	100	0	0.8
	90	100	0	0.8

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

### Detection by fluorescence :

$\lambda_{ex} = 330 \text{ nm}$ ,  $\lambda_{em} = 420 \text{ nm}$

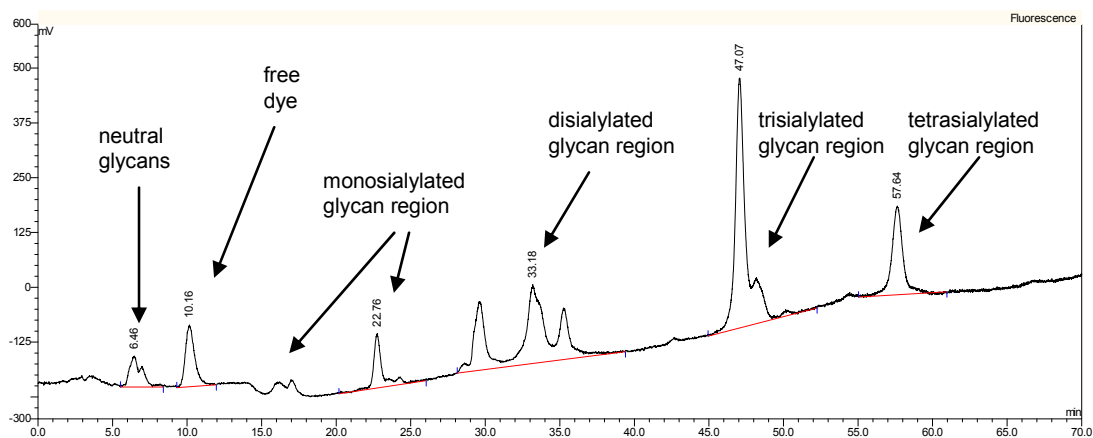


Figure 2: Separation of 2AB labeled glycan standards (picomolar concentration) on LudgerSepC3 7.5 x 75mm using an ammonium formate based solvent.

## Warranties and Liabilities

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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.

## Document Revision Number

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Document # 'LS-C3-Guide', revision v 1.2

## Appendix 1 : Troubleshooting Guide

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Charge mode analysis on LudgerSep C3 is a robust method. If problems do arise they can normally be corrected without difficulty. The following is a guide to the most likely problems, possible causes, and solutions.

### A. Samples are not retained on the column

**1. The glycans are non-anionic.** The glycans do not bear anionic groups or the anionic groups have been removed (e.g. the sample has been desialylated). Minimize desialylation by reducing exposure of the sample to acid conditions and elevated temperatures.

**2. The column may not be fully equilibrated.** Ensure that the column is washed thoroughly in solvent A (20% acetonitrile (aq)) to remove all salts from the column before loading the sample.

**3. The sample contains salt that causes self-elution.** Ensure that the sample is loaded on in water or solvent A and that all traces of salt have been removed.