

Ludger

# LudgerSep™ R1 HPLC Column for Glycan Analysis

## Instruction Guide

Version 2.1

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## LudgerSep R1 Glycan Analysis HPLC Column - Specifications

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<b>Application</b>	Analysis by HPLC of sialic acid variants fluorophore labeled with LudgerTag™ DMB (1,2-diamino-4,5-methylenedioxybenzene.2HCl)		
<b>Description</b>	The R1 HPLC column contains particles with an octadecylsilane coating optimized for hydrophobic chromatography of DMB labeled sialic acids.		
<b>Particles</b>	3 µm silica derivatized with octadecylsilane coating. 120 Angstrom pore size.		
<b>Column Size</b>	<b>Cat #</b>	<b>Diameter x Length</b>	<b>Column Volume</b>
	LS-R1-4.6x150	4.6 x 150 mm	2.49 ml
<b>Column Tube</b>	Stainless steel		
<b>Flow Rates</b>	Typical flow rates = 0.3 – 2.0 ml/min.		
<b>Pressure</b>	Pressure should not exceed 2000psi.		
<b>pH Range</b>	2 - 8		
<b>Temperature</b>	Typical operating temperature = 30 °C, but increasing the temperature may improve resolution for some samples. Maximum temperature range = 15 - 50 °C.		
<b>Solvents</b>	A typical solvent systems for sialic acid analysis is an isocratic gradient of 7:9:84 methanol:acetonitrile:water Avoid strong oxidants and anionic detergents.		
<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 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.		
<b>Suitable Samples</b>	DMB labelled sialic acids		
<b>Sample</b>	Filter samples to 0.2 µm and avoid exposure to light. Ensure DMB labelled samples are kept frozen and out of direct light if they are not to be used immediately.		

**Preparation** Dissolve samples in 7:9:84 methanol:acetonitrile:water.

**Sample Detection** Fluorescence

**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

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LudgerSep R1 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 that 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 that 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.

## Installation

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During column installation we recommend that :

- You should connect the LudgerSep R1 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 R1 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 step is recommended prior to use of the column :

Flush the column sequentially at a flow rate of 1.0 ml/min with 7:9:84 methanol:acetonitrile:water.

## Column Cleaning, Regeneration and Storage

After heavy use, your LudgerSep R1 column may become contaminated with strongly adsorbed sample constituents that will lead to a loss in column performance.

A high acetonitrile solvent will aid removal of hydrophobic compounds. Long (overnight), low flow rate washes at 0.2 ml/min are better than fast (1-2 hour) high flow rate (1 ml/min) washes, for efficient contaminant removal.

The LudgerSep R1 column should be stored in a low aqueous solvent (recommend 80% acetonitrile).

## Sample Preparation

Samples intended for charge mode analysis on LudgerSep R1 columns must be free of salt or anionic detergent and free of any particulates.

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

The DMB labeled sialic acid reference panel (a component of the DMB sialic acid labelling kit, cat. No. LT-KDMB-A1) is an excellent sample to run on this column to ensure efficient performance of the column for sialic acid identification.. An example DMB sialic acid reference panel chromatogram is shown below.

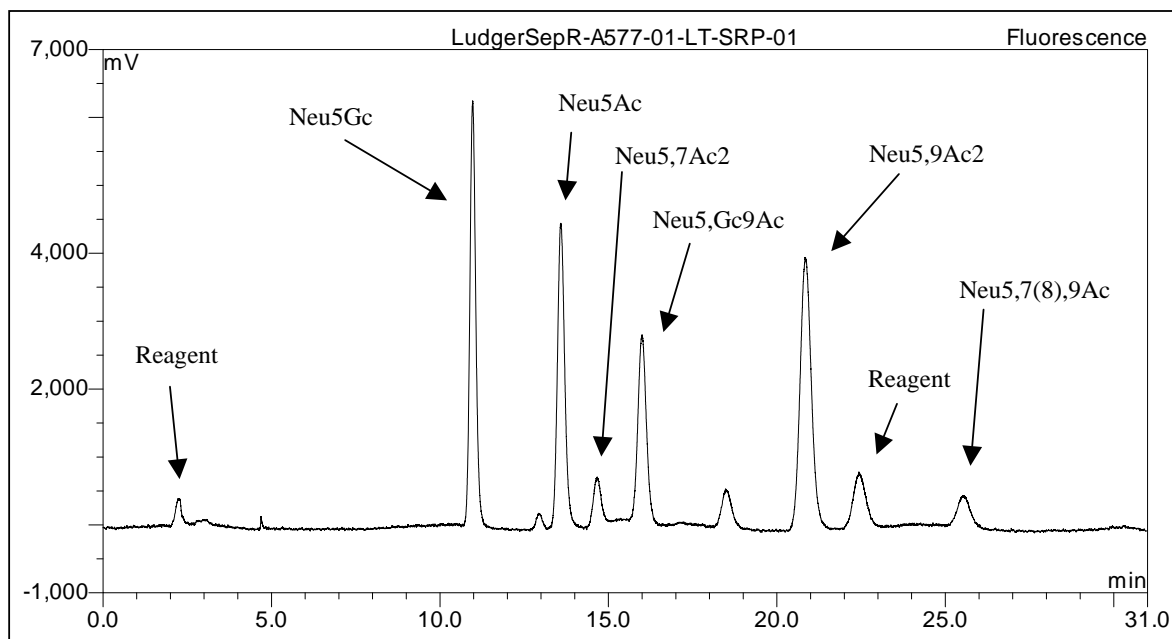


Figure 1: DMB Labeled Sialic Acid Reference Panel Run on the LudgerSep R1 HPLC column.

**Separation Conditions:**

An isocratic gradient at room temperature over a 30 min period is recommended.

Flow rate: 0.5 ml/min

Solvent: Methanol:Acetonitrile:Water (7:9:84)

## 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-R1-Guide', revision v 2.1

## Appendix 1 : Troubleshooting Guide

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DMB labelled sialic acid analysis on LudgerSep R1 is a reasonably 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 column may not be fully equilibrated.** Ensure that the column is washed thoroughly in 7:9:84 methanol:acetonitrile:water.
- 2. The column is overloaded.** Inject a smaller amount of sample to see if retention is improved.
- 3. The column is contaminated.** Clean the column using the methods described in the guide.

### B. Samples are retained and cannot be eluted from the column

- 1. The hplc solvent is not fresh.** Methanol has a low boiling point and will evaporate readily in unsealed bottle. We recommend solvent is prepared on the day of analysis to avoid this problem.