Product Guide for LudgerSep™ R2 HPLC Column for Glycan Analysis

(Ludger Product Code: LS-R2-4.6x150)

Ludger Document # LS-R2-Guide-v1.2

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Specifications for LudgerSep™ R2 Column

Applications Analysis of monosaccharides labeled with 2-aminobenzoic acid (2-AA).

Description The LudgerSep™ R2 HPLC column contains particles with an octadecylsilane coating optimized for hydrophobic chromatography.

Particles 3 μm silica derivatized with octadecylsiliane coating. 175 Angstrom pore size.

Column Size

Cat #	Diameter x Length	Column Volume
LS-R2-4.6x150	4.6 x 150 mm	2.49 ml

Column Tube Stainless steel

Flow Rates Typical flow rates = 0.3 – 2.0 ml/min.

Pressure Pressure should not exceed 2000psi.

pH Range 2 - 8

Temperature Typical operating temperature = 30 °C, but increasing the temperature may improve resolution for some samples.
Maximum temperature range = 15 - 50 °C.

Solvents Solvent A: 0.2% butylamine/ 0.5% phosphoric acid/ 1% tetrahydrofuran in purified water.
Solvent B: Acetonitrile
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.
Long term storage of the column should be in solvent containing at least 30% acetonitrile.

Suitable Samples Monosaccharides labeled with 2-aminobenzoic acid (2-AA)

Sample	Filter samples to 0.2 μm and avoid exposure to light.
Preparation	Dissolve samples in Solvent A.
Sample Detection	Fluorescence. Excitation: 360 nm. Emission: 425 nm.
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

The LudgerSep™ R2 column 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 of the Column

During column installation we recommend that :

- You should connect the LudgerSep™ R2 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 R2 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 step is recommended prior to use of the column :

For 2-AA monosaccharide analysis flush the column at a flow rate of 0.8 mL/min with BPT solvent for 10 minutes. Increase the acetonitrile concentration to 50% over 5 minutes and hold at a 0.8 mL/min flow rate for 20 minutes. Return the solvent to 100% solvent A, over 5 minutes, and keep at this flow rate for 20 minutes before first using the column for sample analysis.

Column Cleaning and Storage

After heavy use, your LudgerSep™ R2 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 (1ml/min) washes, for efficient contaminant removal.

The LudgerSep™ R2 column should be stored in a low aqueous solvent. We recommend acetonitrile (minimum 30%) but other solvents such as isopropanol can also be used.

Sample Preparation

Samples for injection onto the LudgerSep™R2 column should be free of particulates. Particulates can be removed from samples using microcentrifuge filters with 0.2 μm pore size membranes.

Analysis of 2-AA labeled Monosaccharides

For release of monosaccharides from glycoproteins and labeling with 2-aminobenzoic acid (2-AA), please follow the guide provided with the LudgerTag™ Monosaccharide release and Labeling Kit, (Cat. No. LT-MONO-96).

The LudgerSep™R2 column gives very good separation of the seven main monosaccharides found in most N-link and O-link glycans. Samples are diluted into the butylamine/ortho-phosphoric acid/tetrahydrofuran (BPT) HPLC running solvent.

Solvents

The glycan analysis gradients are based on the following solvents:

Solvent A : purified water based solvent containing 0.2 % butylamine (2 mL per litre), 0.5 % phosphoric acid 5 mL per litre), 1 % tetrahydrofuran (10 mL per litre) (henceforth called BPT solvent).

Solvent B : 50 % acetonitrile : 50 % solvent A

We have also tested acetonitrile-free solvent systems. This requires a slightly different gradient. Please enquire for further details.

Gradient

Column temperature: 30°C

Fluorescence detector settings : Excitation wavelength: 360 nm, Emission wavelength: 425 nm

Time (min)	%B	Flow Rate (ml/min)
0.00	7	0.8
7.00	7	0.8
25	17	0.8
26	100	0.8
36	100	0.8
37	7	0.8
45	7	0.8

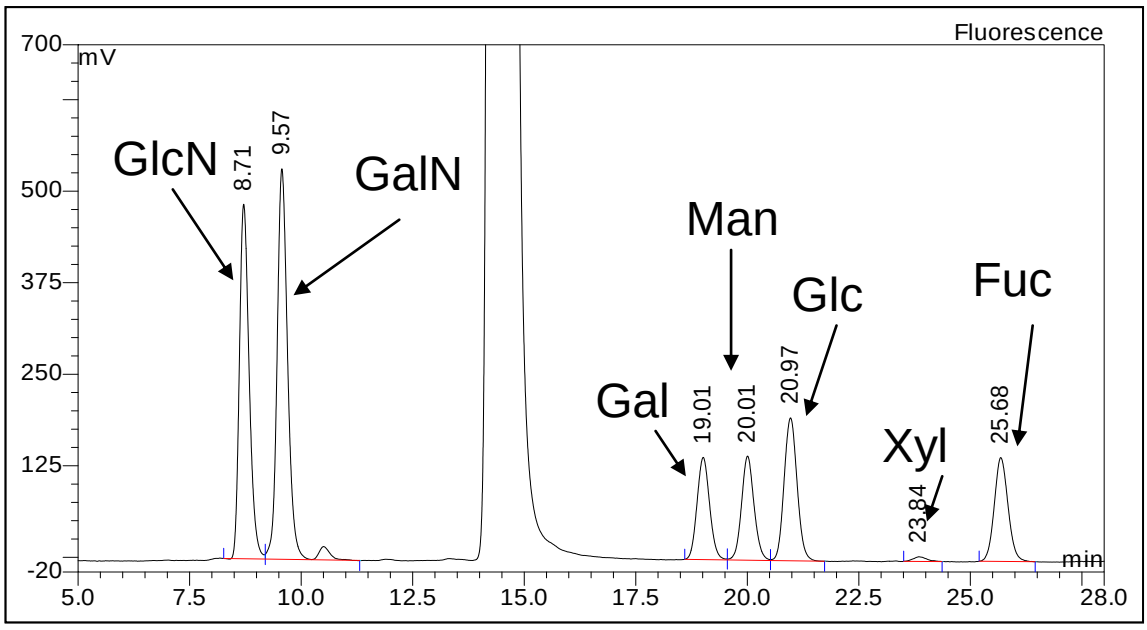

Figure 1: 2-AA fluorescence chromatogram of monosaccharides glucosamine (GlcN), galactosamine (GalN), galactose (Gal), mannose (Man), glucose (Glc), xylose (Xyl) and fucose (Fuc).

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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Appendix 1 : Troubleshooting Guide

Analysis of 2-AA labeled monosaccharides using the LudgerSep R2 is a reasonably robust method. If problems do arise they can usually 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 or elute early.

1. The column may not be fully equilibrated. Ensure that the column is washed thoroughly in Solvent A before running samples.
2. The solvent has gone off. The chromatography is sensitive to accurate levels of each solvent component. For example, low levels of butylamine will cause peaks to elute early and cause the free dye peak (usually around 15 min elution time) to elute earlier, possibly co-eluting with the amino monosaccharides.
3. The column is contaminated. Perform an overnight wash of the column in 70% acetonitrile at a low flow rate (about 0.2 mL/min).

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

1. The HPLC solvent is not fresh. Check that the acetonitrile levels are correct. Whereas, GlcN and GalN will elute without acetonitrile, the neutral monosaccharides will not.

C. The monosaccharides/free dye have different retention times to a previous column.

1. Solvent variability. Check that solvents are prepared methodically for each batch.

2. Column variability. Whilst every effort is made to ensure each column resin batch is the same, some variations may occasionally occur. Before columns are available for purchase we test them with a 2-AA monosaccharide mix standard to produce a CofA. A certain amount of variation is acceptable, but if separation performance is lower than expected, please contact Ludger and we will assist to remedy the situation.