



**Product Guide for LudgerSep™ uR2  
UHPLC Column  
for Monosaccharide Analysis**

**(Ludger Product Code: LS-UR2-2.1x50)**

**Ludger Document # LS-uR2-Guide-v1.2**

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## Specifications for LudgerSep™ uR2 Column

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<b>Applications</b>	Analysis of monosaccharides labeled with 2-aminobenzoic acid (2-AA) using UHPLC.		
<b>Description</b>	The LudgerSep™ uR2 UHPLC column contains particles with an endcapped octadecylsilane coating optimized for hydrophobic chromatography.		
<b>Particles</b>	1.9 µm silica derivatized with octadecylsilane coating. 175 Angstrom pore size.		
<b>Column Size</b>	<b>Cat #</b>	<b>Diameter x Length</b>	<b>Column Volume</b>
	LS-UR2-2.1x50	2.1 x 50 mm	0.17 ml
<b>Column Tube</b>	Stainless steel		
<b>Flow Rates</b>	Typical flow rates = 0.1 – 1.0 ml/min.		
<b>Pressure</b>	Pressure should not exceed 15000psi. Higher pressure applications result in reduced column lifespan.		
<b>pH Range</b>	1 - 11		
<b>Temperature</b>	Typical operating temperature = 35 °C, but increasing the temperature may improve resolution for some samples. Maximum temperature range = 15 - 60 °C.		
<b>Solvents</b>	Solvent A: 0.2% butylamine/ 0.5% phosphoric acid/ 1% tetrahydrofuran in purified water (A pre-prepared x10 concentrate of this solvent is available from Ludger, catalogue number LS-R-BPTX10). Solvent B: Acetonitrile 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. Long term storage of the column should be in solvent containing at least 30% acetonitrile.		

<b>Suitable Samples</b>	Monosaccharides labeled with 2-aminobenzoic acid (2-AA)
<b>Sample</b>	Filter samples to 0.2 $\mu\text{m}$ and avoid exposure to light. For best resolution of chromatogram peaks use a full loop injection method with a sample loop of 5 $\mu\text{l}$ volume or less.
<b>Preparation</b>	Dissolve samples in Solvent A.
<b>Sample Detection</b>	Fluorescence. Excitation: 360 nm. Emission: 425 nm.
<b>Handling:</b>	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.
<b>Safety:</b>	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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The LudgerSep™ uR2 column is designed for use with the latest generation of UHPLC instruments capable of withstanding high flow pressures and fast sample analyses. In order to take advantage of the high resolving power of sub 3  $\mu\text{m}$  particle size containing columns, we recommend keeping sample injection volumes below 5  $\mu\text{L}$  and minimising system void volumes. Ideally use full loop injection with a sample loop of less than 5  $\mu\text{L}$ . Tubing should be narrow bore (about 0.13 mm diameter or less) and detector flow cell volumes should be 10  $\mu\text{l}$  or less. Although an example chromatogram is shown in this guide, retention times will vary dependent on the UHPLC system used.

## Installation of the Column

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During column installation we recommend that :

- You should connect the LudgerSep™ uR2 column to your HPLC system using standard 0.13 mm ID tubing and zero dead volume connectors. 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 sample dispersion effects.
- Ensure that all solvents are filtered prior to use. A pre-prepared solvent concentrate is available from Ludger, Catalogue Number LS-R-BPTX10.
- Before analysing any samples, the newly installed column should be conditioned using the protocol described below.

## Preconditioning of the Newly Installed Column

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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 0.4 mL/min with BPT solvent for 10 minutes. Increase the acetonitrile concentration to 50% over 1 minute and hold at a 0.4 mL/min flow rate for 10 minutes. Return the solvent to 100% solvent A, over 1 minute, and keep at this flow rate for 10 minutes before first using the column for sample analysis.

## Column Cleaning and Storage

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After heavy use, your LudgerSep™ uR2 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 0.1 ml/min are better than fast (1-2 hour) high flow rate (0.5 ml/min) washes, for efficient contaminant removal.

The LudgerSep™ uR2 column should be stored in a low aqueous solvent. We recommend acetonitrile (minimum 30%) but other solvents such as isopropanol can be used.

## Sample Preparation

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Samples for injection onto the LudgerSep™uR2 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

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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™uR2 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 and Gradient

The glycan analysis gradients are based on the following solvents. We recommend using these as a guide to developing the most appropriate gradient for your HPLC system.

#### 8 Minute Separation

An 8 minute chromatographic separation is recommended for HPLC systems that have pressure limits of up to 600 bar. It is not recommended to try this separation on an HPLC that is not ultra high pressure rated.

**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). A pre-prepared solvent x10 concentrate is available from Ludger, Catalogue Number LS-R-BPTX10.

**Solvent B** : 100 % acetonitrile

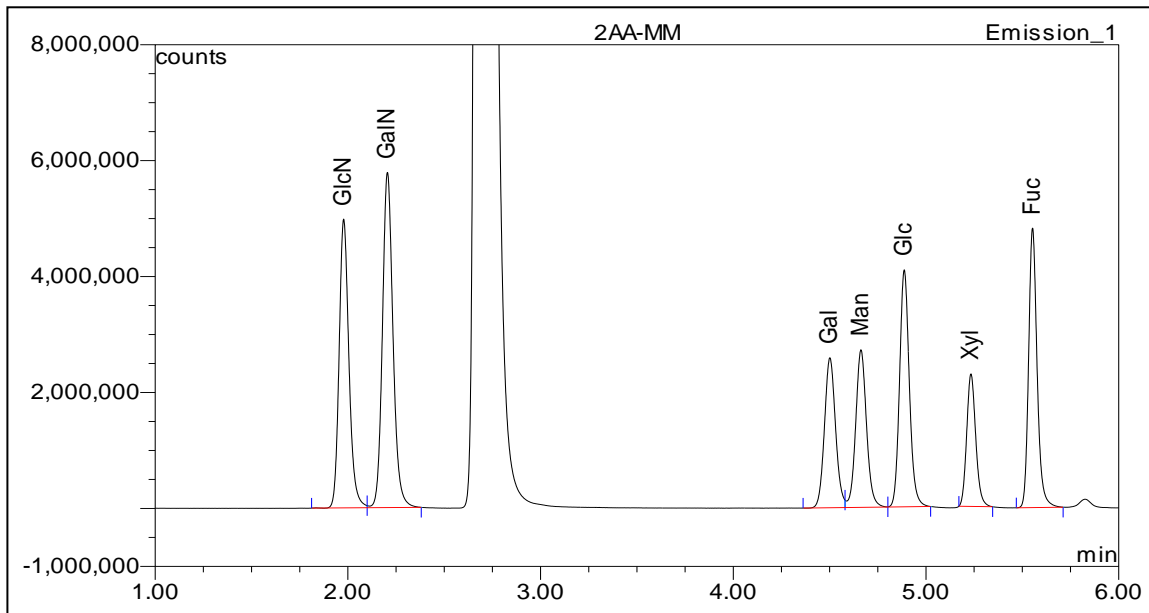
**Column temperature:** 35°C

**Typical backpressure:** 250-440 Bar

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

**Gradient:**

Time (min)	%B	Flow Rate (ml/min)
0.0	0.0	0.4
1.0	0.0	0.4
4.5	15.7	0.4
4.6	50.0	0.4
5.6	50.0	0.4
6.0	0.0	0.6
8.0	0.0	0.6



**Figure 1: 8 minute 2-AA fluorescence chromatogram of monosaccharides glucosamine (GlcN), galactosamine (GalN), galactose (Gal), mannose (Man), glucose (Glc), xylose (Xyl) and fucose (Fuc).**

## Related Glycoanalysis Products

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Description	Cat #	Usage / Notes
LudgerSep™R2	LS-R2-4.6x150	Analytical HPLC version of the LS uR2 column for HPLC systems that cannot operate at pressures in excess of 200 bar. Column dimensions are 4.6x150 mm and particle size is 3.0 µm.
LudgerTag™ 2-AA Monosaccharide Release and Labeling Kit	LT-MONO-96	For release and labeling of neutral and amino monosaccharides from glycoprotein therapeutics and pre-released glycans. This kit can be used for full quantitative or routine monosaccharide analysis.
LudgerSep™ R BPT solvent	LS-R-BPTX10	A prepared solvent concentrate containing butylamine, ortho-phosphoric acid and tetrahydrofuran for running up to 130 solvent gradients on the uR2 UHPLC column.



## 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 warranties, 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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## Appendix 1 : Troubleshooting Guide

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Analysis of 2-AA labeled monosaccharides using the LudgerSep uR2 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 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 0.1 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 elute as broad peaks and free dye complexes will not elute.

## **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. 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.