LudgerSep™ R1 HPLC Column for 2-AB Labeled Glycan Analysis

Instruction Guide

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LudgerSep R1 Glycan Analysis HPLC Column - Specifications

Application	Analysis by HPLC of 2-aminobenzamide (2-AB) labeled glycans[1]. Two columns joined with a zero dead volume connector are required for this application.		
Description	The R1 HPLC column contains particles with an octadecylsilane coating optimized for hydrophobic chromatography of 2-AB labelled glycans.		
Particles	3 µm silica derivatized with octadecylsiliane coating. 120 Angstrom pore size.		
Column Size	**Cat #**	**Diameter x Length**	**Column Volume**
	2 x LS-R1-4.6x150	4.6 x 150 mm	4.98 ml in total
Column Tube	Stainless steel		
Flow Rates	Typical flow rates = 0.3 ml/min.		
Pressure	Pressure should not exceed 2000psi.		
pH Range	2 - 8		
Temperature	Typical operating temperature = 30 °C. Maximum temperature range = 15 - 50 °C.		
Solvents	A typical solvent systems for 2-AB glycan analysis is 0.1% acetic acid for solvent A, and 0.1% acetic acid in an aqueous solution containing 10% 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.		
Suitable Samples	2-AB labelled glycans.		
Preparation	Dissolve samples in 0.1% acetic acid or water, and inject in a volume not exceeding 50 µl. Long term storage (e.g. several days) of sialylated glycans at room temperature		

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could result in partial desialylation, but in general it is recommended that samples are prepared immediately prior to use. Five pmols of sample with up to 5 glycans will be readily detectable using most standard fluorometers.

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

LudgerSep R1 columns can be used with any HPLC pumping system capable of delivering accurate gradients at a flow rate of 0.3 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). Larger injection volumes, up to 50 μl, may result in peak broadening and volumes over 50 μl are not recommended.

Installation

During column installation we recommend that :

- You should connect the two LudgerSep R1 columns 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. A zero-dead volume connector should be used to connect the two columns.
- 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 first LudgerSep R1 column to prevent damage to the column by particles.
- Before analysing any samples, the newly installed columns should be conditioned using the protocol described below.

Column Preconditioning

The following preconditioning step is recommended prior to use of the column :

Flush the column sequentially at a flow rate of 0.3 ml/min with 0.1% acetic acid and then gradually ramp the solvent over 2 minutes to 0.1% acetic acid in 10% acetonitrile.

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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 0.1 ml/min are better than fast (1-2 hour) high flow rate (0.5 ml/min) washes, for efficient contaminant removal.

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

Sample Preparation

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

An example chromatogram of 2-AB labelled antibody glycans is shown in Figure 1.

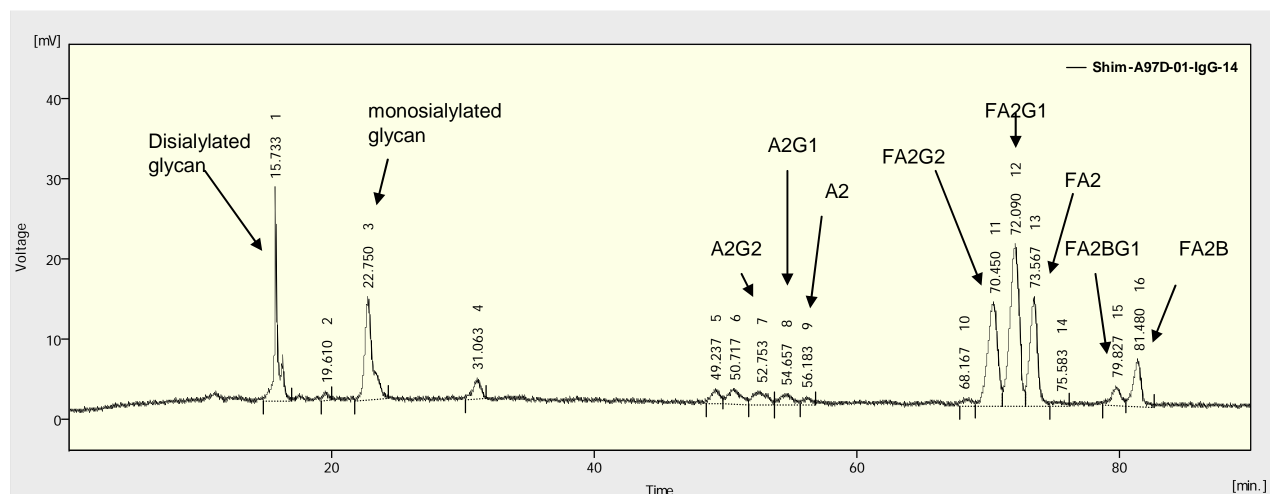

Figure 1: 2-AB labelled human IgG immunoglobulin glycans on 2 x LudgerSep R1 HPLC columns.

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Separation Conditions:

Temperature:	30 °C
Flow rate:	0.3 mL/min increasing up to 0.5 mL/min for wash stage
Solvent A:	0.1% acetic acid in hplc grade water.
Solvent B:	0.1% acetic acid in 10:90 acetonitrile:hplc grade water mix.

Gradient:

Time (min)	%B
0	50 – 0.3 mL/min
5.0	50 – 0.3 mL/min
70.0	85 – 0.3 mL/min
75.0	85 – 0.5 mL/min
80.0	50 – 0.5 mL/min
90.0	50 – 0.5 mL/min

Glycan Retention Times

Glycan - Oxford Nomenclature[2] (alternative names in brackets)	Retention Time (min)
MAN3 (M3N2)	55.2
MAN5	50.2
MAN6	44.7
MAN7	43.1
MAN8	40.3
MAN9	39.9
A2 (NGA2/G0)	57.2
A2G1 (G1)	55.7
A2G2 (NA2/G2)	53.8
A2G2S1 (A1)	16.7
A2G2S2 (A2)	10
FA2 (NGA2F/G0F)	73.6
FA2G1 (G1F)	72.2
FA2G2 (NA2F/G2F)	69.6
FA2G2S1 (A1F)	22.2
FA2G2S2 (A2F)	11.5
A3 (NGA3)	57.9
A3G3 (NA3)	54.7
A3G3S3 (A3)	9.8
A4 (NGA4)	51.5
A4G4 (NA4)	46.1
M5A1B (Hybrid)	51.6

Table 1: Retention times of 2-AB labeled glycans on the 2 x LudgerSep R1 HPLC column system. Values are given as a guide only. Expect different retention times on other hplc systems, and with alternative glycan isomers.

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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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Reference

1. Chen, X. and Flynn, G.C. “Analysis of N-glycans from recombinant immunoglobulin G by on-line reversed-phase high-performance liquid chromatography/mass spectrometry” Analytical Biochemistry, 370 (2007), 147-161.

2. Harvey DJ, Merry AH, Royle L, Campbell MP, Dwek RA and Rudd PM. “Proposal for a standard system for drawing structural diagrams of N- and O-linked carbohydrates and related compounds.” Proteomics, 15 (2009), 3796-3801.

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Appendix 1 : Troubleshooting Guide

2-AB glycan analysis on LudgerSep R1 columns 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 0.1% acetic acid solution.

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 but the retention time varies

1. The solvent preparation may vary from batch to batch. Ensure that the acetic acid and acetonitrile components are accurately and consistently measured.

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