

Ludger™

LudgerSep™ N1 Amide HPLC Column for Glycan Analysis

Instruction Guide

Ludger Limited

Culham Science Centre
Abingdon
Oxfordshire OX14 3EB
United Kingdom

T: +44 870 085 7011

F: +44 870 163 4620

E: info@ludger.com

W: www.ludger.com

Contents

	Pages
LudgerSep™ N1 Glycan Analysis HPLC Column - Specifications	3
Glycan Analysis with the N1 Amide HPLC Column	5
Warranties and Liabilities	6
Document Revision Number	6

LudgerSep N1 Glycan Analysis HPLC Column - Specifications

Application	Analysis and purification by HPLC of LudgerTag™ fluorophore and UV -chromophore labeled glycans.			
Description	The N1 HPLC column contains particles with a polymeric amide coating optimized for high resolution chromatography of complex glycan mixtures.			
Particles	5 µm particle size with 80 angstrom pores and polymeric amide coating.			
Column Size	Cat #	Description	Dimensions	Col Vol
	LS-N1-4.6x250	LudgerSep N1 HPLC Column	4.6 x 250 mm	4.2 ml
	LS-N1-4.6x10	LudgerSep N1 Guard Column	4.6 x 10 mm	0.17 ml
Flow Rates	Typical flow rates = 0.4 - 1.0 ml/min. Maximum flow rate = 1.2 ml/min			
Column Pressure	Maximum pressure = 2250 psi (150 kg/cm ²)			
pH Range	2.0 - 7.5			
Temperature	Typical operating temperature = 30 °C. Maximum temperature range = 10 - 80 °C.			
Solvents	Typical solvent systems for glycan analysis include gradients of acetonitrile(aq) and buffers containing ammonium formate, pH 4.4 .			
Shipping Solvent	75% acetonitrile - 25% water			
Cleaning Solvents	<ol style="list-style-type: none"> 1. Water [to remove very polar solutes from the bonded phase] 2. 45% acetonitrile (aq) [to desorb hydrophobic compounds] 3. 0.1% triethylamine in 80% acetonitrile [to remove desorbed basic compounds] 4. 50 mM ammonium formate pH 4.4 / acetonitrile (1:1 v/v) [to remove ionic compounds] 			
Storage	Before long-term storage flush the column with at least 5 column volumes of 75% acetonitrile (aq).			

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.

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 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-AA (2-aminobenzoic acid), 2-AB (2-aminobenzamide), AMAC (2-aminoacridone), 2-AP (2-aminopyridine), AMC (7-Amino-4-methylcoumarin), ABEE (4-Aminobenzoic ethyl ester).

Sample Filter samples to 0.2 µm then dry using a centrifugal evaporator.

Preparation Re-dissolve in 5 - 50 µl of the starting buffer (i.e. the solvent mixture used at the very start of the HPLC gradient) then inject. If possible, inject the sample soon after dissolution to minimise problems with sample precipitation in high organic solvent conditions.

Sample Detection Either fluorescence or UV -absorbance depending on the dye used (see the appropriate LudgerTag instruction guide).

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 - 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

Glycan Analysis with the N1 Amide HPLC Column

Solvents

The glycan analysis gradients in this guide are based on the following solvents :

Solvent A : Acetonitrile

Solvent B : 50 mM ammonium formate pH 4.4

Gradient # N1-01 : General-Purpose Oligosaccharide Analysis Gradient

Use as a general-purpose gradient for analysis of LudgerTag 2-AB and 2-AA labeled oligosaccharides.

Time (min)	% A	%B	Flow Rate (ml/min)
0	65	35	0.4
75	50	50	0.4
80	0	100	0.4
83	0	100	0.4
85	65	35	0.4
115	65	35	0.4

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.

Document Revision Number

Document # 'LS-N1-Guide', revision v 1.1