Product Guide for LudgerSep™ N1 amide HPLC Columns for Glycan Analysis

(Ludger Product Codes: LS-N1-4.6x10 and LS-N1-4.6x250)

Ludger Document # LS-N1-Guide-v2.1

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Specifications for LudgerSep™ N1 Columns

Application — Analysis and purification by HPLC of LudgerTag™ fluorophore and UV-chromophore labeled glycans.

Description — LudgerSep™ N1 HPLC colums contain 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	Volume
LS-N1-4.6x10	LudgerSep™ N1 Guard Column	4.6 x 10 mm	0.17 ml
LS-N1-4.6x250	LudgerSep™ N1 HPLC Column	4.6 x 250 mm	4.2 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. This is available from Ludger and can be diluted for use. Cat No. LS-N-BUFFX40

Shipping Solvent — 75% acetonitrile - 25% water

Cleaning Solvents

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).

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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 1pmol - 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 Preparation Filter samples to 0.2 μm then dry using a centrifugal evaporator.
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

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HPLC System Requirements

LudgerSep N1 columns can be used with an HPLC system capable of delivering accurate gradients at a flow rate of 0.3 to 1.0 ml/min. In general, systems which 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.

For the 4.6mm column inject the sample in up to 100 µl of the starting buffer (i.e. the solvent mixture used at the very start of the HPLC gradient).

A fluorescence detector is required with the following detection wavelengths:

Fluorescence Label		λ_{ex} (nm)	λ_{em} (nm)
2-AB	[2-aminobenzamide]	330	420
2-AA	[2-aminobenzoic acid]	330	420
AA-Ac	[3-(acetylamino)-6-aminoacridine]	442	525

For optimal detection, use wide slit widths (e.g. 10 – 20 nm). Sub-picomole levels of 2-AB or 2-AA labelled glycans can be detected with good signal-to-noise (depending on the sensitivity of the detector used).

To improve repeatability and intermediate precisions for glycan analyses use a column temperature controller. Good results can be obtained with a column temperature of 35°C.

Installation of the Column

During column installation we recommend that :

- You should connect the LudgerSep N1 column to your HPLC system using standard 1/16" OD tubing and 10-32 (1/16") fittings in either stainless steel or PEEK (polyetheretherketone). Finger-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 0.2 µm filter with minimal dead volume between the injector and the head of the LudgerSep N1 column to prevent damage to the column by particles.
- Before analysing any samples, condition your newly installed column as follows.

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Preconditioning of the Newly Installed Column

Prepare your newly installed column for glycan analysis as follows:

- Precondition the column by running two complete LSN1-01 gradients (see below) without any sample injected.
- Check the column performance by running a fluorescently labeled glucose homopolymer (GHP) as a system suitability standard using a LSN1-01 gradient.

Column Cleaning and Storage

After heavy use, your LudgerSep N1 column may become contaminated with strongly adsorbed sample constituents that will lead to a loss in column performance.

Light Contamination

1 Clean the column following the protocol in the 'Column Preconditioning' section above.
2 Equilibrate in the starting buffer for the LSN1-01.
3 Do a blank run without injecting a sample to check the baseline.

Cleaning Medium Contamination

1 Run through one LSN1-01 gradient.
2 Wash with 50% (v/v) 50 mM ammonium formate pH 4.4 cat. No. LS-N-BUFFX40, 50% (v/v) acetonitrile at 0.1 ml/min overnight
3 Run through one LSN1-01 gradient
4 Run a system suitability check

Cleaning Heavy Contamination

Wash the column with the following:

1 Water [4 h or more at 0.1 ml/min] - *to remove very polar solutes from the bonded phase*
2 45% acetonitrile (aq) [4 h or more at 0.1 ml/min] - *to desorb hydrophobic compounds*
3 0.1% triethylamine in 80% acetonitrile [4 h or more at 0.1 ml/min] - *to remove desorbed basic compounds*
4 50 mM ammonium formate pH 4.4 / acetonitrile (1:1 v/v) [4 h or more at 0.1 ml/min] - *to remove ionic compounds*
5 Run through one LSN1-01 gradient.
6 Run a system suitability check

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Long Term Storage

For long-term storage, the column should be washed with a gradient going from the operational buffer to 100% water then to the storage solvent of >50% acetonitrile in water (v/v).

Sample Preparation

The N1 column should be used for analysis of purified glycans (fluorescently labeled or unlabeled). Samples must be free of particulates. The sample should be dissolved in water **before** addition of acetonitrile to make up to the same composition as the start of the gradient (e.g. dissolve sample in 35 µl water, then add 65 µl acetonitrile before injection to run with the N-glycan analysis gradient method: LSN1-01)

Fluorescent Labeling

Fluorescent glycans can be prepared by derivatizing pure glycans using reductive amination with a LudgerTag label and purification on a LudgerClean cartridge. The following are commonly used labeling and purification systems for biopharmaceutical glycosylation analysis:

Labeling System	Post-Labeling Purification System
2-AB (LudgerTag™ kit # LT-KAB-A2)	LudgerClean™ S-Cartridge (Cat # LC-S-A6)
2-AA (LudgerTag™ kit # LT-KAA-A2)	LudgerClean™ S-Cartridge (Cat # LC-S-A6)
AA-Ac (LudgerTag™ kit # LT-KAAAC-A2)	LudgerClean™ D1-Cartridge (Cat # LC-D1-A6)

Filtering Samples

Remove particulates from samples by filtering through a spin filter or syringe filter with 0.2 µm pore size membrane.

Sialylated Glycans

Sialylated glycans can become desialylated if exposed to acidic conditions and elevated temperatures. Avoid desialylation with such samples by

a. minimizing exposure to acid (if possible, keep the pH between 5 - 8), and
b. minimizing exposure to temperatures greater than 25°C.

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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 Cat. No. LS-N-BUFFX40

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

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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

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