



Ludger

LudgerClean™ D1

Glycan Cleanup Cartridges

Product Guide

Ludger Ltd

Culham Science Centre

Abingdon

Oxfordshire OX14 3EB

United Kingdom

Tel: +44 870 085 7011

Fax: +44 870 163 4620

Email: ludgerclean@ludger.com

www.ludger.com

Contents

	Pages
LudgerClean™ D1 Glycan Cleanup Cartridges - Specifications	3
Additional Reagents and Equipment Required	4
Introduction	5
Time Line for Cleanup	5
Protocol A: For Cleanup of AA-Ac Labeled N- and O-Glycans	6 - 7
Protocol B: For Cleanup of 2-AB or 2-AA Labeled N-Glycans	8 - 9
Warranties and Liabilities	10
Document Revision Number	10
Material Safety Data Sheet	11

LudgerClean D1 Glycan Cleanup Cartridges - Specifications

Part Number **LC-D1-x-Ay** where x = milligrammes of resin and y = number of cartridges in pack (e.g. LC-D1-30-A6 is a kit of 6 cartridges eaching containing 30 mg resin)

Description The cartridges contain an unique solid phase extraction (SPE) resin that binds a wide range of fluorescently labeled glycans and allows purification of these from labeling reagents.

Application For post-labeling purification of LudgerTag fluorophore and chromophore labeled glycans. Suitable for cleanup after glycan labeling with 2-AB (2-aminobenzamide), 2-AA (2-aminobenzoic acid), and AA-Ac [3-(acetylamino)-6-aminoacridine].



LudgerTag D1 cartridges before and after binding of LudgerTag AA-Ac dye.

Using the wash Protocol A, labeled glycans are eluted from the cartridge while the orange colored dye stays bound to the resin.

Binding Capacity Each LC-D1 cartridge can typically bind up to 2 μg of fluorescently labeled O- or N-linked glycans.

Number of Samples The LudgerClean D1 cartridges are designed for single use only.

Suitable Samples A wide range of glycans can be purified. These include N-linked and O-linked type oligosaccharides, tri-saccharides and larger structures. Two protocols are provided for the following types of samples:

Protocol A: for purification of glycans after AA-Ac labeling

Protocol B: for purification of N-glycans after 2-AB or 2-AA labeling

- Structural Integrity** No detectable (< 2 mole per cent) loss of sialic acid, fucose, sulfate, or phosphate.
- Binding Selectivity** Essentially stoichiometric binding and elution for most complex glycan mixtures.
- Storage:** Store at room temperature in the dark. Protect from sources of heat, light, and moisture. The cartridges are stable for at least two years as supplied.
- Shipping:** The product can be shipped at ambient temperature.
- 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 hazardous 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

Additional Reagents and Equipment Required

Reagents

- Pure water (HPLC grade)
- Acetonitrile (HPLC grade)
- Wash A (for cleanup after AA-Ac labeling): 20% acetonitrile, 80% water (v/v)
- Wash B (for cleanup of N-glycans after 2-AB or 2-AA labeling): 96% acetonitrile, 4% water (v/v)

Equipment

- Pipettes
- 0.5 μm or 0.2 μm microcentrifuge filters
- Microcentrifuge

Introduction

LudgerClean D1 cartridges have been designed for purification of glycans from non-carbohydrate material including salts, proteins, and detergents. Applications include cleanup of glycans following hydrazinolysis, endoglycosidase digests (including PNGase F digests), and enzyme treatment, and before and after fluorescent labeling.

There are two D1 cartridge protocols:

- Protocol A: for purification of glycans after AA-Ac labeling
- Protocol B: for purification of N-glycans after 2-AB or 2-AA labeling

Time Line for Cleanup

The LudgerClean D1 glycan cleanup procedure typically takes around 65 minutes :

Procedure	Time	Elapsed Time (minutes)
Filter samples	20 min	20
Wash and prime cartridges	15 min	25
Apply sample	10 min	35
Wash off non-glycan contaminants	15 min	50
Elute glycans	15 min	65

LudgerClean D1 Protocol A: For Cleanup for AA-Ac Labeled N- and O-Glycans

Outline of Method

The outline of the cleanup procedure is as follows :

- **Wash and prime the cartridge**

Wash then prime the LudgerClean D1 cartridge by washing with acetonitrile then water.

- **Prepare then apply glycan sample**

Dilute out any organic solvents from the sample with water.

Apply the aqueous solution of glycan sample to the cartridge.

- **Wash off the non-glycan contaminants**

Wash non-glycan contaminants such as salts with water.

- **Elute the glycans**

Elute AA-Ac labeled glycans using Wash A (20% acetonitrile, 80% water, v/v)

- **Post elution workup**

Filter the eluted labeled glycan solution.

- **Analyse the glycans**

The glycans are now ready for analysis.

Protocol A

1 Wash and prime the cartridge

Prepare each LudgerClean D1 cartridge by washing with the following:

Reagent	Volume (ml)
Acetonitrile	2
Water	2

This removes any impurities that may have bound to the resin matrix during storage and prepares the

surface of the resin for binding of labeled glycans.

If the flow is restricted, e.g. by an air gap, then apply a slight pressure to the top of the cartridge (e.g. using a clean, gloved thumb) in order to resume normal flow.

N.B. The D1 cartridges can be used with gentle air pressure or gentle vacuum to push or pull washes through the resin bed and increase the speed of washing.

2 Prepare the glycan samples

Dissolve each sample from the AA-Ac labeling reaction with 500 µl water

3 Apply the samples to the cartridge

Load each sample onto a primed cartridge.

AA-Ac labeled glycans should bind to the matrix while salts and other hydrophilic non-glycan contaminants pass through.

4 Wash off non-glycan contaminants

Wash the cartridge with 2 x 0.5 ml water

This washes residual salts and hydrophilic non-glycan material off the column.

5 Elute the glycans

Place the cartridge over a collection vessel and recover the glycans by eluting with 4 x 0.5 ml of Wash A (20% acetonitrile, 80% water, v/v) allowing each aliquot to drain before the next is applied.

AA-Ac labeled glycans should be eluted while hydrophobic non-glycan material (including excess AA-Ac dye) remain bound to the solid phase matrix.

6 Dry the eluted glycans (optional)

If appropriate, evaporate the glycan containing fraction to dryness, then redissolve in a desired volume of water or solvent for further analysis.

Samples should be dried if the acetonitrile in the elution solvent could interfere with subsequent analysis.

7 Filter the eluted glycans

Filter samples using a microcentrifuge spin filter with a PTFE or polypropylene membrane with pores less than 0.5 µm .

Protocol B: Outline of D1 Cleanup for 2-AB and 2-AA Labeled N-Glycans

The outline of the cleanup procedure is as follows :

- **Prime the cartridge**

Prime the active surface of the resin by washing with Wash B (96% acetonitrile, 4% water, v/v) then acetonitrile.

- **Apply the glycan sample**

Dissolve each glycan sample with 0.5 ml Wash B then load to the cartridge and leave to absorb onto the resin.

- **Wash off the non-glycan contaminants**

Wash out non-glycan material (e.g. excess labeling dye) with Wash B.

- **Elute the glycans**

Elute the labeled glycans using Wash A.

- **Post elution workup**

Filter the eluted glycan solution.

- **Analyse the glycans**

The glycans are now ready for analysis.

Protocol B

1 Wash and prime the cartridge

Prepare each LudgerClean D1 cartridge by washing with the following:

Reagent	Volume
Wash B (96 % acetonitrile, 4% water, v/v)	2 x 1 ml
Acetonitrile	1 ml

This removes any impurities that may have bound to the resin matrix during storage and prepares the surface of the resin for binding of labeled glycans.

If the flow is restricted, e.g. by an air gap, then apply a slight pressure to the top of the cartridge (e.g. using a clean, gloved thumb) in order to resume normal flow.

N.B. The D1 cartridges can be used with gentle air pressure or gentle vacuum to push or pull washes through the resin bed and increase the speed of washing.

2 Apply the samples to the cartridge

Dilute each sample with 0.5 ml Wash B (96 % acetonitrile, 4% water) and vortex to mix the sample.

Apply each sample to the top of a cartridge and allow the solvent to flow through the cartridge.

2-AB or 2-AA labeled glycans should bind to the matrix while salts and other non-glycan contaminants pass through.

3 Allow sample to adsorb onto membrane

Wait for 15 minutes to let the sample adsorb onto the matrix.

4 Wash off non-glycan contaminants

Wash the cartridge with each of the following:

- 1 ml acetonitrile
- 5 x 1 ml Wash B (96 % acetonitrile, 4% water)

This washes residual salts and non-glycan material off the column.

5 Elute the glycans

Place the cartridge over a collection vessel and recover the glycans by eluting with 2 x 0.5 ml of Wash A (20% acetonitrile, 80% water, v/v) allowing each aliquot to drain before the next is applied.

2-AB or 2-AA labeled glycans should be eluted while hydrophobic non-glycan material remain bound to the solid phase matrix.

6 Dry the eluted glycans (optional)

If appropriate, evaporate the glycan containing fraction to dryness, then redissolve in a desired volume of water or solvent for further analysis.

Samples should be dried if the acetonitrile in the elution solvent could interfere with subsequent analysis.

7 Filter the eluted glycans

Filter samples using a microcentrifuge spin filter with a PTFE or polypropylene membrane with pores less than 0.5 μm

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 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.

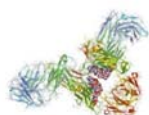
This product is intended for *in vitro* research only.

Document Revision Number

Document # 'LC-D1-30-Ax-Guide' , version 3.1

Material Safety Data Sheet

Manufacturer	Ludger Ltd Culham Science Centre, Oxford OX14 3EB, UK Tel: +44 870 085 7011, Fax: +44 870 163 4620 Email: safety@ludger.com, Website: www.ludger.com
Identification of the substance	LudgerClean D1 cartridges
Composition	Tube of polypropylene containing glycan absorption resin
Hazard identification	Non hazardous.
First aid measures	In case of contact: Eyes: irrigate with plenty of water. Skin: wash with soap and water. Ingestion: drink plenty of water. Inhalation: move to a well ventilated area and clear nose and throat. If in doubt seek medical advice.
Fire fighting measures	Non hazardous. Water spray or appropriate foam according to surrounding fire conditions.
Accidental release measures	Wash spill site with copious amounts of water.
Handling and storage	Store at room temperature. Handle in accordance with Good Laboratory Practice.
Exposure Controls /	Wear appropriate protective clothing (safety spectacles, gloves, laboratory coat) in accordance with Good Laboratory Practice.
Physical and chemical properties	Constructed of solid plastic and polymeric materials
Stability and reactivity	Not combustible.
Toxicological information	Toxicological, carcinogenic and mutagenic properties have not been investigated.
Ecological information	Data not available.
Disposal considerations	No special requirements. Dispose of according to local requirements.
Transport information	Contact Ludger Ltd for transportation information.
Regulatory information	Data not available.
Other information	The advice offered is derived from the currently available information on the hazardous materials in this product or component. Consideration has been made regarding the quantities offered in the pre-dispensed container. The advice offered is, therefore, not all inclusive nor should it be taken as descriptive of the compound generally.



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Certificate of Conformity

LudgerClean D1 Glycan Cleanup Cartridges

Cat. # : LC-D1-30-A6

Lot # : A78G-05

Size : 6 cartridges per kit

This kit conforms to the specifications given in Ludger document # LC-D1-x-Guide.

Each kit contains the following components :

Quantity per Kit	Cat #	Lot #	Component Name
6	LC-D1-30-A6-cartridge	A78G-05	LudgerClean D1 cartridge