



Product Guide for LudgerClean™ D1

Glycan Cleanup Cartridges

(Ludger Product Code: LC-D1-30-Ax where x denotes pack size)

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Specifications for LudgerClean™ D1 Cartridges

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

Description For post-labeling purification of LudgerTag™ fluorophore and chromophore labeled glycans. Suitable for cleanup after glycan labeling with AA-Ac [3-(acetylamino)-6-aminoacridine]. This cartridge also works with 2-AB and 2-AA fluorophores, but for these applications we recommend LC-A-24 cartridges for higher recovery rates.



LudgerClean™ D1 cartridges before and after binding of LudgerTag™ AA-Ac dye.
(left cartridge = before use, right cartridge = after elution of glycans).

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

Number of Samples 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.

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 solution (for cleanup after AA-Ac labeling): 20% acetonitrile, 80% 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.

Timeline for Cleanup

The LudgerClean™ D1 glycan cleanup procedure typically takes around 75 minutes :

Procedure	Time	Elapsed Time (minutes)
Filter samples	20 min	20
Wash and prime cartridges	15 min	35
Apply sample	10 min	45
Wash off non-glycan contaminants	15 min	60
Elute glycans	15 min	75

Instructions for Use

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 removes residual salts and hydrophilic non-glycan material from the column.

Discard these washes into a suitable waste container.

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 solution (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 .

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

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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™ D 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.