



LudgerClean™ EB10

Glycan Cleanup Cartridges

Product Guide

Ludger Document # LC-EB10-Ax-Guide version 2.2

Ludger Ltd

Culham Science Centre, Oxford OX14 3EB, United Kingdom

Tel: +44 870 085 7011, Fax: +44 870 163 4620

Email: ludgerclean@ludger.com

www.ludger.com

Contents

	Pages
LudgerClean™ EB10 Glycan Cleanup Cartridges - Specifications	3
Additional Reagents and Equipment Required	4
Introduction	5
Outline of LudgerClean EB10 Cleanup Protocol	5
Time Line for Cleanup	6
Preparation of Cleanup Cartridges	7
Application of Sample and Removal of Contaminants	7
Elution of Glycans	8
Warranties and Liabilities	10
Document Revision Number	10
Material Safety Data Sheet	11

LudgerClean EB10 Glycan Cleanup Cartridges - Specifications

Application For purification of glycans from a variety of complex mixtures (including removal of salts and detergents).

Description The cartridges contain an unique non-porous Electronic Interaction (EI) matrix. This acts like a super-hydrophobic resin that binds even very hydrophilic glycans. Most salts and detergents either simply pass through the cartridges or bind very lightly and can be washed off before the glycans are eluted.



Binding Capacity Each LC-EB10 cartridge can typically bind up to 50 µg of O- or N-linked glycans.

Number of Samples The LudgerClean EB10 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. The cartridges are **not** suitable either for monosaccharides or disaccharides which are generally bound too weakly for efficient purification or for large linear poly-sialylated glycans which can be bound very tightly to the resin. Glycan samples must be applied to the cartridges in solutions that are substantially aqueous.

Structural Integrity No detectable (< 2 mole per cent) loss of sialic acid, fucose, sulfate, or phosphate.

Binding efficiency > 95 % for most glycans

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

- Pure water (HPLC grade)
- Methanol
- 1M sodium hydroxide (aq)
- 30% acetic acid (aq)
- Acetonitrile (HPLC grade)
- Trifluoroacetic acid (Analar grade)
- Wash A: 5 % (v/v) acetonitrile (aq) plus 0.1% trifluoroacetic acid
- Wash B: 50 % (v/v) acetonitrile (aq) plus 0.1% trifluoroacetic acid
- Pipettes
- 0.5 µm or 0.2 µm microcentrifuge filters
- Microcentrifuge

Introduction

LudgerClean EB10 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.

Outline of LudgerClean EB10 Cleanup Protocol

The outline of the cleanup procedure is as follows :

- **Wash the cartridge**

The LudgerClean EB10 cartridge is washed with successive washes of water, 1 M sodium hydroxide, water, 30% acetic acid, then water.

- **Prime the resin**

The active surface of the resin in the cartridge is primed by washing with acidic aqueous solutions of acetonitrile.

- **Prepare the glycan sample**

Dilute out any organic solvents and remove viscous or particulate material from the sample.

- **Apply the glycan sample**

The aqueous solution of glycan sample is applied to the cartridge.

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

Non-glycan contaminants such as salts and detergents are washed out using dilute acidic aqueous acetonitrile.

- **Elute the glycans**

Bound glycans are washed off the cartridge using a higher concentration of acidic aqueous acetonitrile.

- **Post elution workup**

The eluted glycan solution is filtered.

- **Analyse the glycans**

The glycans are now ready for analysis.

Time Line for Cleanup

The LudgerClean EB10 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

Preparation of Cleanup Cartridges

1 Wash the cartridge

Prepare each LudgerClean EB10 cartridge by washing with the following:

Reagent	Volume (ml)
methanol	0.5
1M sodium hydroxide	0.5
water	1
30% acetic acid	1
water	1

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

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. Do not force liquid through the resin bed either by applying excessive pressure or by using a vacuum as these can cause blockage of the porous frits or leakage of resin through the bottom frit.

2 Prime the cartridge

Prime each cartridge with the following:

Reagent	Volume (ml)
Wash B	0.5
Wash A	1

This prepares the surface of the resin for binding of the glycans.

Application of Sample and Removal of Contaminants

3 Prepare the glycan sample

The sample to be cleaned must be in either an aqueous buffer or one containing only a low percentage of organic solvent. If the sample contains organic solvent then dilute with water until the organic solvent content is less than 5 % by volume.

Some types of sample may contain particulate or viscous material that can block the flow through the EB10 cartridges. These include some glycoproteins subjected to glycan release by endoglycosidase treatment. Monoclonal antibodies treated with PNGase F are particularly prone to formation of viscous material. In such cases, to minimize blockage of the EB10 cleanup cartridges:

- a. Dilute each sample with 500 µl water.
- b. Mix thoroughly by vortexing.
- c. Spin in a micro-centrifuge (typical conditions are centrifugation at 10,000 rpm for 15 minutes).
- c. Carefully pipette out the supernatant and apply to the prepared EB10 cartridge (see step 4).

Centrifugation is best done in 1.5 ml or 2 ml polypropylene microcentrifuge tubes.

4 Apply the sample

Apply the sample to the cartridge.

Glycans should bind to the matrix while salts and non-hydrophobic non-glycan contaminants pass through.

5 Wash off non-glycan contaminants

Wash the cartridge with the following:

Reagent	Volume (ml)
water	0.7
Wash A	0.7

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

Elution of Glycans

6 Elute the glycans

Place the cartridge over a collection vessel and recover the glycans by eluting with 4 x 0.2 ml of Wash B allowing each aliquot to drain before the next is applied.

Glycans should be eluted while hydrophobic material such as certain peptides, proteins, and detergents remain bound to the solid phase matrix.

7 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.

8 Filter the eluted glycans

Samples should be filtered to at least 0.5 μm .

Filtration is best done using microcentrifuge spin filters with an inert membrane – e.g. PTFE or polypropylene.

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.

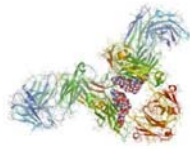
This product is intended for *in vitro* research only.

Document Revision Number

Document # 'LC-EB10-Ax-Guide' , version 2.2

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 EB10 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.



Ludger

Certificate of Conformity

LudgerClean EB10 Cartridges

Cat. # : LC-EB10-A6

Lot # : A7CC-02

Size : 6 cartridges per kit

This kit conforms to the specifications given in Ludger document # LC-EB-Ax Guide.

Each kit contains the following components :

Quantity per Kit	Cat #	Lot #	Component Name
6	LC-EB10-A6	A7CC-02	LudgerClean EB10 cartridge



Certificate of Conformity

LudgerClean EB10 Cartridges

Cat. # : LC-EB10-A6

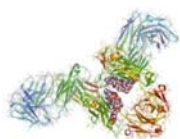
Lot # : A814-01

Size : 6 cartridges per kit

This kit conforms to the specifications given in Ludger document # LC-EB-A6 Guide.

Each kit contains the following components :

Quantity per Kit	Cat #	Lot #	Component Name
6	LC-EB10-A6	A814-01	LudgerClean EB10 cartridge



Ludger

Certificate of Conformity

LudgerClean EB10 Cartridges

Cat. # : LC-EB10-A6

Lot # : A82K-01

Size : 6 cartridges per kit

This kit conforms to the specifications given in Ludger document # LC-EB-A6 Guide.

Each kit contains the following components :

Quantity per Kit	Cat #	Lot #	Component Name
6	LC-EB10-A6	A82K-01	LudgerClean EB10 cartridge



Certificate of Conformity

LudgerClean EB10 Cartridges

Cat. # : LC-EB10-A6

Lot # : A83D-11

Size : 6 cartridges per kit

This kit conforms to the specifications given in Ludger document # LC-EB-A6 Guide.

Each kit contains the following components :

Quantity per Kit	Cat #	Lot #	Component Name
6	LC-EB10-A6	A83D-11	LudgerClean EB10 cartridge