

Product Guide for
LudgerClean™ EB10
Glycan Cleanup
Cartridges

Product # LC-EB10-A6

Ludger Document # LC-EB10-Ax-Guide-v5.1

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Specifications for LudgerClean™ EB10 Cartridges



Application	For purification of glycans from a variety of complex mixtures (including removal of salts and detergents).
Description	The cartridges contain a 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 LudgerClean™ EB10 cartridge can typically bind up to 50 µg of O- or N-linked glycans.
Number of Samples	LudgerClean™ EB10 cartridges are designed for single use only.
Suitable Samples	<p>A wide range of glycans can be purified. These include N-linked and O-linked type oligosaccharides, tri-saccharides and larger structures.</p> <p>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.</p> <p>Glycan samples must be applied to the cartridges in solutions that are substantially aqueous.</p>
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, plastic ware 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 Safety Data Sheet (SDS) 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), enzyme treatment, and before and after fluorescent labeling.

Outline of LudgerClean™ EB10 Cleanup Protocol

1 Wash the cartridge

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

2 Prime the cartridge

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

3 Prepare the glycan sample

Dilute out any organic solvents and filter if required to remove viscous or particulate material from the sample.

4 Apply the glycan sample

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

5 Wash off the non-glycan contaminants

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

6 Elute the glycans

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

7 Dry the eluted glycans (optional)

The eluted glycan solution can now be concentrated if required.

8 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

Instructions for Use

Preparation of Cartridges

1 Wash the cartridge



Prepare each LudgerClean™ EB10 cartridge by washing with the following:

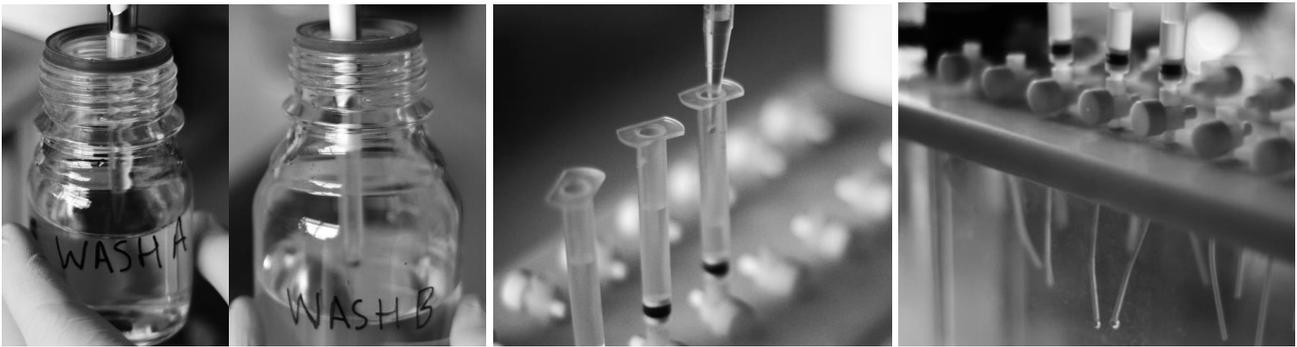
Reagent	Volume (ml)
methanol	0.5
1M sodium hydroxide	0.5
water	1
30% acetic acid	1
water	1

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 μ l 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).

We recommend that centrifugation is performed using 1.5 ml or 2 ml polypropylene microcentrifuge tubes.

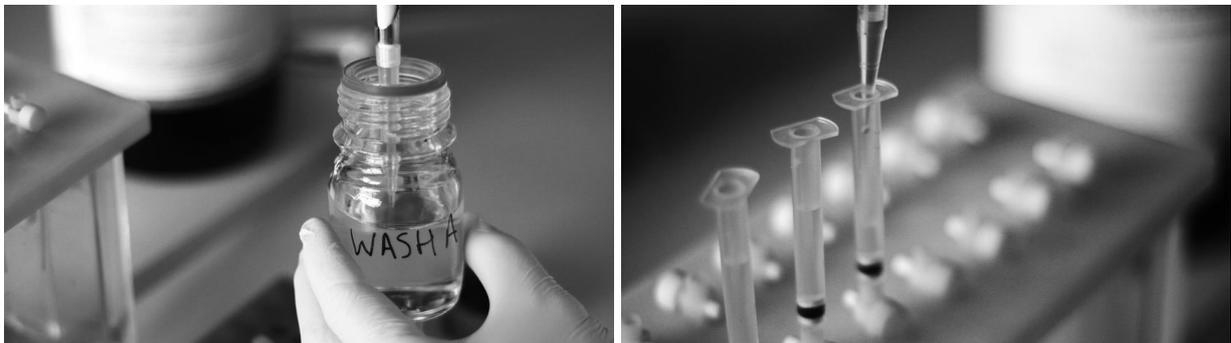
4 Apply the glycan sample



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

Discard these washes into a suitable waste container.

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 Analyse the glycans

The glycans are now ready for analysis. Typical methods include 2AB or procainamide labeling.



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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SAFETY DATA SHEET

Version: 1.1

 Date written/revised: 16th April 2019

SECTION 1. IDENTIFICATION OF THE SUBSTANCE/PREPARATION AND OF THE COMPANY / UNDERTAKING

Product Name	LudgerClean EB10 cartridges
Product Catalogue Name	LC-EB10-A6
CAS-No.	7440-44-0
Company:	Ludger Ltd Culham Science Centre Abingdon Oxfordshire OX14 3EB
Telephone:	01865 408554
Emergency Telephone:	01865 408554
Email:	info@ludger.com

SECTION 2. HAZARDS IDENTIFICATION

2.1 Classification of the substance or mixture

Classification according to Regulation (EU) No. 1272/2008 [EU-GHS/CLP]

This product has been classed as a non-hazardous substance or mixture according to Regulation (EC) No. 1272/2008.

2.2 Label elements

The product does not require any labelling in accordance with EC directives or respective national laws.

Signal Word: None.

Hazard Statement(s)

None.

Precautionary Statement(s)

None.

2.3 Other hazard information:

None.

SECTION 3. COMPOSITION/INFORMATION ON INGREDIENTS

3.1 Substances

Synonyms:	Carbon, Charcoal activated
Formula:	C
Molecular weight:	12.01 g/mol

Component		Concentration
Name	Carbon	-
CAS-No.	7440-44-0	
EC-No.	None.	

SECTION 4. FIRST AID MEASURES

4.1 Description of first aid measures

General Advice

Consult a physician if exposure causes ill effects and if in any doubt. Show this safety data sheet to the physician/ first responder in attendance.

If Ingested

Rinse mouth well with water, if person is conscious. Do not give anything by mouth if unconscious.

If skin is exposed

Wash the affected area well with soap and water.

If eyes are exposed

Rinse eyes with water/ eye wash solution for at least 5 minutes as a precaution. If safe and easy to do so remove contact lenses and continue rinsing.

If inhaled

Move affected person to a source of ventilation/ fresh air. If not breathing give artificial respiration.

4.2 Most important symptoms and effects, both acute and delayed

To the best of our knowledge, the chemical, physical and toxicological properties have not been fully investigated.

4.3 Indication of immediate medical attention and special treatment needed

No data available.

SECTION 5. FIRE-FIGHTING MEASURES

5.1 Extinguishing media

Select extinguishing media appropriate to surrounding area, compatible media's are water spray, alcohol-resistant foam, dry chemical or carbon dioxide.

5.2 Special hazards arising from the substance or mixture

No data available.

5.3 Advice for firefighters

Wear self-contained breathing equipment, if necessary.

SECTION 6. ACCIDENTAL RELEASE MEASURES

6.1 Personal precautions, protective equipment and emergency procedures

Avoid formation of dust and breathing it in. Wear PPE.

6.2 Environmental Precautions

None required.

6.3 Methods and material for containment and cleaning up

Use a damp cloth to sweep up the spilled product. Put the contaminated material and waste product into a suitable container with a lid and arrange disposal.

6.4 Reference to other sections

See Section 13 for more information on disposal.

SECTION 7. HANDLING AND STORAGE

7.1 Precautions for safe handling

Handle the product wearing PPE, when used as part of clean up use under extraction, due to the nature of the chemicals used in the process, not the product itself.

7.2 Conditions for safe storage, including any incompatibilities

Store in a dry, well-ventilated area. Keep sealed in container until required preventing contamination.

7.3 Specific end uses

No data available.

SECTION 8. EXPOSURE CONTROLS/PERSONAL PROTECTION

8.1 Control parameters

Components with workplace control parameters

This product contains no substances with occupational exposure limit values.

8.2 Exposure controls

Appropriate engineering controls

Handle the product wearing Personal protective equipment, wash and dry hands before and after handling the product. General good laboratory and safety practice.

Personal Protective Equipment

Eye / face protection

Wear eye protective equipment tested and approved under appropriate government standards such as NIOSH (US) or EN 166 (EU).

Skin protection

Handle the product wearing gloves. Gloves are to satisfy the specifications of EU Directive 89/686/EEC and the standard EN 374 derived from it. Gloves to be checked for tares/holes before use and to be removed using the proper glove removal technique, so that the outer side of the gloves do not touch any skin. Gloves are to be disposed of as contaminated solid waste. See Section 13 for information on waste disposal.

Body Protection

Wear a laboratory coat or similar covering over the operators outside clothing.

Respiratory protection

Respiratory protection is not required with this product (on its own), when used for clean up the chemicals used require that the product is handled under extraction.

Thermal hazards

None.

SECTION 9. PHYSICAL AND CHEMICAL PROPERTIES

9.1 Information on basic physical and chemical properties

Appearance	Form: fine powder
	Colour: black
Odour	Odourless
Odour threshold	None
pH	6.0 – 9 at 40g/l at 25°C
Freezing/Melting Point	Melting point/range: 3,550°C – lit.
Initial boiling point and boiling range	No data available
Flash Point	No data available
Evaporation rate	No data available
Flammability	No data available

Upper/lower flammability or explosive limits	No data available
Vapour Pressure	<0.01 hPa at 20°C
Vapour Density	No data available
Relative Density	0.250 – 0.600 g/cm ³
Solubility in water	Insoluble
Partition coefficient	No data available
Autoignition temperature	No data available
Decomposition temperature	No data available
Viscosity	No data available
Explosive properties	No data available
Oxidising properties	No data available

9.2 Other information

Bulk Density	250 – 550 kg/m ³ at 20°C
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SECTION 10. STABILITY AND REACTIVITY

10.1 Reactivity

No data available.

10.2 Chemical stability

The product is stable at the correct storage conditions.

10.3 Possibility of hazardous reactions

No data available.

10.4 Conditions to Avoid

High moisture and extreme temperatures.

10.5 Incompatible materials

Strong oxidizing agents.

10.6 Hazardous decomposition products

Other decomposition products.

SECTION 11. TOXICOLOGICAL INFORMATION

11.1 Information on toxicological effects

Acute toxicity

LD₅₀ Intravenous – Mouse – 440 mg/kg

Skin corrosion/irritation

No data available.

Serious eye damage/irritation

No data available.

Respiratory or skin sensitisation

No data available.

Germ cell mutagenicity

No data available.

Carcinogenicity

IARC: No component of this product presents at levels greater than or equal to 0.1% is identified as probable, possible or confirmed human carcinogen by IARC.

Reproductive toxicity

No data available.

STOT-single exposure

No data available.

STOT-repeated exposure

No data available.

Aspiration hazard.

No data available.

Potential Health Hazards**Inhalation**

Inhalation

May be harmful if inhaled. May cause irritation to the respiratory tract.

Ingestion

Ingestion

May be harmful if swallowed.

Skin

Skin

May be harmful if absorbed through skin. May cause irritation to the skin.

Eyes

May causes eye irritation.

Signs and symptoms of exposure

To the best of our knowledge the chemical, physical and toxicological properties have not been thoroughly investigated.

Additional Information

RTECS: FF5250100

SECTION 12. ECOLOGICAL INFORMATION**12.1 Toxicity**

No data available.

12.2 Persistence and degradability

No data available.

12.3 Bioaccumulative potential

No data available.

12.4. Mobility in soil

No data available.

12.5. Results of PBT and vPvB assessment

No data available.

12.6. Other adverse effects

No data available.

SECTION 13. DISPOSAL CONSIDERATIONS**13.1 Waste treatment methods**

Dispose of by using a licensed professional chemical liquid and solid waste disposal company. To be incinerated.

Contaminated packaging

Dispose of packaging as solid contaminated waste.

SECTION 14. TRANSPORT INFORMATION**14.1 UN Number**

