多糖纯化柱图文指南

Product Guide for

LudgerClean™ EB10 Glycan Cleanup Cartridges

Product # LC-EB10-A6

Ludger Document # LC-EB10-Ax-Guide-CN-v4.0

Ludger Ltd

Culham Science Centre

Oxford OX14 3EB

United Kingdom

Tel: +44 1865 408 554

Fax: +44 870 163 4620

Email: info@ludger.com

www.ludger.com

Contents

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	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), enzyme treatment, and before and after fluorescent labeling.

Outline of LudgerClean™ EB10 Cleanup Protocol

1 清洗柱子 Wash the cartridge

LudgerClean™ EB10 柱子是用水, 1 M 氢氧化钠 , 水, 30% 醋酸, 最后再用水，来进行接替冲洗。

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

2 预备纯化柱 Prime the cartridge

柱子里树脂的活性表面是用酸性的乙腈水溶液进行洗涤。

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

3 准备多糖样品 Prepare the glycan sample

把样品上任何有机溶剂稀释掉并且过滤以去除样品中的任何粘性或颗粒状物质。

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

4 添加多糖样品 Apply the glycan sample

把多糖样品的水溶液加入到柱子里。

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

5 清洗非聚糖的污染物 Wash off the non-glycan contaminants

用稀酸性的乙腈水溶液来洗掉非聚糖的污染物如盐, 洗涤剂等。

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

6 洗脱多糖 Elute the glycans

用高酸性的乙腈水溶液从柱子上洗脱吸附的多糖。

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

7 干燥洗脱下来的聚糖(可选) Dry the eluted glycans (optional)

如需,洗脱后的多糖可浓缩。

The eluted glycan solution can now be concentrated if required.

8 多糖分析 Analyse the glycans

聚糖可以进行分析了。

The glycans are now ready for analysis.

Time Line for Cleanup

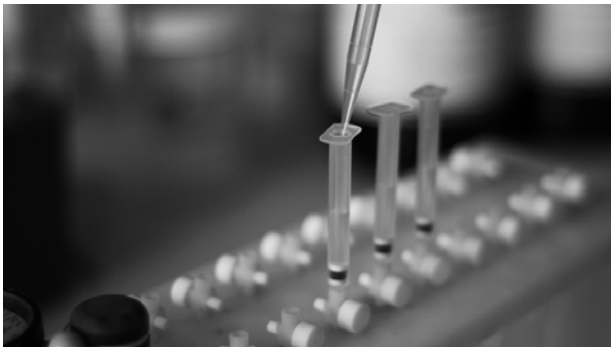
The LudgerClean™ EB10 glycan cleanup procedure typically takes around 65 minutes :

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

使用说明 Instructions for Use

柱子的准备 Preparation of Cartridges

1 清洗纯化柱 Wash the cartridge



用以下试剂清洗每一个 LudgerClean™ EB10 纯化柱

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

试剂 **Reagent**	容积 **Volume (ml)**
甲醇 methanol	0.5
氢氧化钠 1M sodium hydroxide	0.5
水 water	1
醋酸 30% acetic acid	1
水 water	1

这样能清除在储存期间任何可能粘附到树脂基上的杂质。

如果流量受阻，例如被气隙堵塞，可以在柱子的顶部施加轻微的压力（如用干净，戴着手套的拇指）以恢复正常流动。

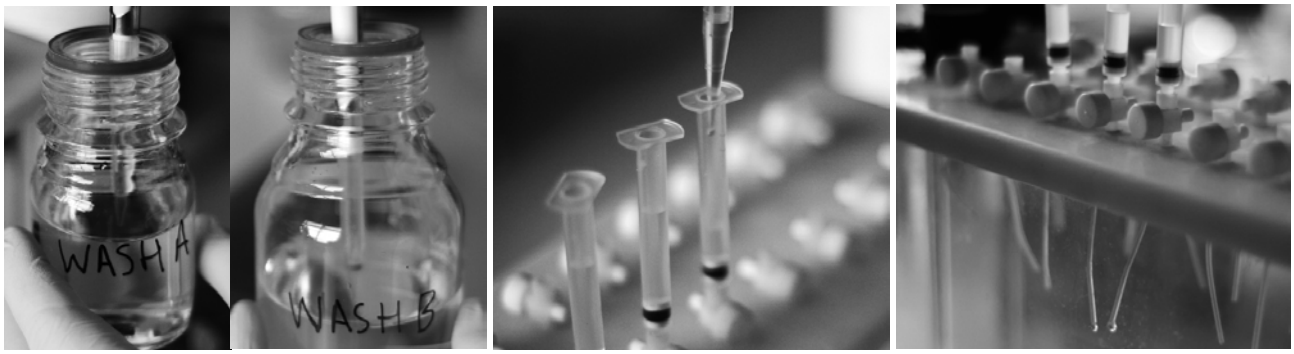
This removes any impurities that may have bound to the resin matrix during storage.

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.

不要使用过度压力或通过抽真空等此类的操作强制使液体流过树脂床，这样会导致多孔柱筛堵塞或树脂从底部柱筛的泄漏。

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 预备纯化柱 Prime the cartridge



用以下方式预备纯化柱

Prime each cartridge with the following:

试剂 Reagent	容积 Volume (ml)
清洗 B Wash B	0.5
清洗 A Wash A	1

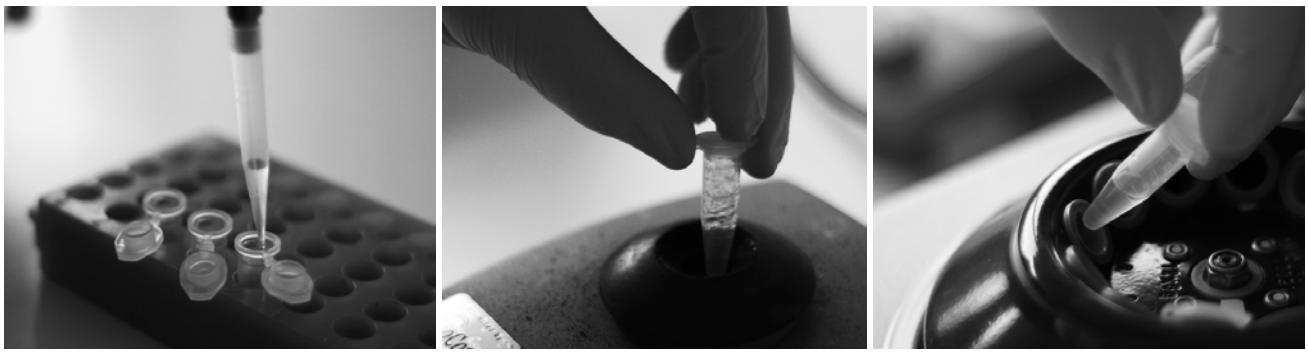
这是为树脂表面与聚糖的粘附作准备。

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

加入样品和杂质去除

Application of Sample and Removal of Contaminants

3 多糖样品的制备 Prepare the glycan sample



要清理的样品必须溶入在缓冲溶液或含有低含量的有机溶剂里。如果样本包含有机溶剂，那就用水稀释到有机溶剂含量小于 5%。

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.

某些类型的样品可能含有微粒或粘性物质，而这些物体是会阻止样品经过 EB10 柱子的流动。这包括一些经过内切糖苷酶处理过的糖蛋白样品。单克隆抗体用 PNGase F 处理后特别容易形成粘性物质。在这种情况下，以便减少 EB10 清洁柱的堵塞:

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. 用 500 毫升水稀释每个样本。 Dilute each sample with 500 µl water.
b. 用涡流彻底地混合。 Mix thoroughly by vortexing.
c. 用微型离心机分离样品(典型条件是要 10000 转的离心转 15 分钟)。
   Spin in a micro-centrifuge (typical conditions are centrifugation at 10,000 rpm for 15 minutes).
d. 小心地吸取出上清液，然后将上清液涂在制备好的 EB10 柱子上 (见步骤 4)。
   Carefully pipette out the supernatant and apply to the prepared EB10 cartridge (see step 4).

我们建议进行离心时使用 1.5 ml 或 2 ml 的聚丙烯微型离心管。

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

4 添加多糖样品 Apply the glycan sample

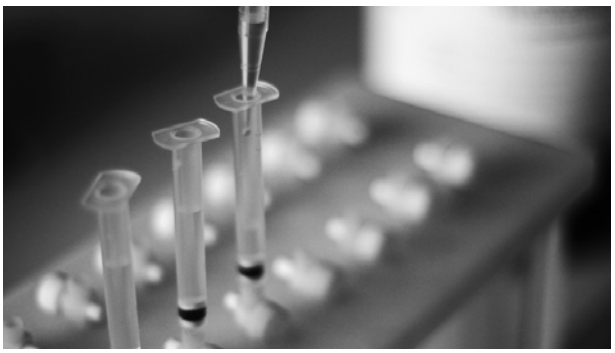

将样品加到柱子里

Apply the sample to the cartridge.

聚糖会粘附到基质上，而盐和非疏水非聚糖污染物则会流出。

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

5 清洗非聚糖的污染物 Wash off the non-glycan contaminants



用以下方式清洁纯化柱

Wash the cartridge with the following:

试剂 Reagent	容量 Volume (ml)
水 Water	0.7
洗液 A Wash A	0.7

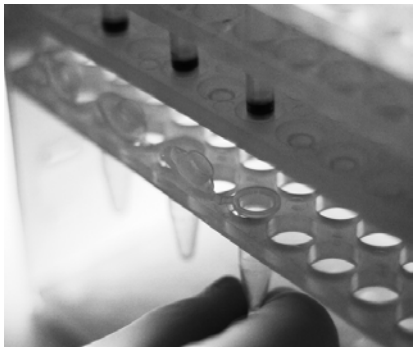
这样从柱子上清除了残余的盐和非疏水非聚糖物质。将这些洗涤丢弃到合适的废容器中。

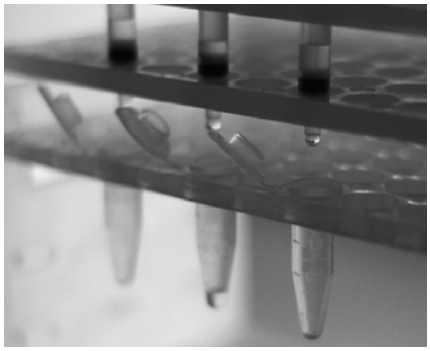
This removes residual salts and non-hydrophobic non-glycan material from the column.

Discard these washes into a suitable waste container.

洗脱聚糖 Elution of Glycans

6 洗脱聚糖 Elute the glycans



把柱子放到收集容器上, 然后通过 4 x 0.2 ml 的 B 清洗液来洗脱以回收聚糖。在加入下一次洗液之前，确保每等份的洗液充分流出。

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.

聚糖会被洗脱出来，而疏水性材料，如某些多肽、蛋白质、和洗涤剂应仍然粘附在固相基质上。

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

7 干燥洗脱下来的聚糖(可选) Dry the eluted glycans (optional)

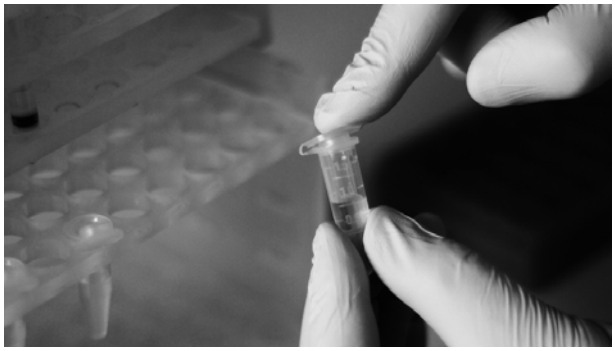
如果适宜, 蒸发洗脱的聚糖至干燥状态, 然后溶于一定体积的水或溶剂为进行下一步分析。

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

8 多糖分析 Analyse the glycans

聚糖可以进行分析了。通常这包括 2AB 或普鲁卡因胺标签

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 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-CN-v4.0

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 indentification	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.
Toxilogical 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.**