Ludger

Product Guide for LudgerClean™ CEX Cartridges

(Ludger Product Code: LC-CEX-A6)

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Contents

Specifications for LC-CEX-A6

Application	For purification of O-glycans from non-carbohydrate material including salts, proteins, and detergents. Applications include cleanup of glycans following hydrazinolysis or any other non-reductive O-glycan release method.
Description	The cartridges contain a cation exchange resin that is stored in 1 M hydrochloric acid.
Binding capacity	Each LudgerClean™ CEX cartridge can typically clean up O-glycans released from 1000 µg of glycoprotein.
Number of Samples	LudgerClean™ CEX cartridges are designed for single use only.
Suitable Samples	A wide range of O-glycans can be purified including di-saccharides, tri-saccharides and oligosaccharides. Glycan samples must be applied to the cartridges in solutions that are substantially aqueous.
Structural Integrity	No detectable loss of sialylated glycans.
Binding Selectivity	Essentially stoichiometric binding and elution for most complex glycan mixtures.
Storage:	Store refrigerated at 4 to 10°C in the dark. Protect from sources of heat, light, and moisture. The cartridges are stable for at least 6 months from date of manufacture.
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: resistivity 18 MΩ-cm, particle free (>0.22 μm), TOC <10 ppb
- Pipettes
- pH paper
- Centrifugal evaporator (e.g.ThermoSavant SpeedVac® or GeneVac®).

Outline of LudgerClean™ CEX Cleanup Protocol

1 Prepare the glycan sample

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

2 Prime the cartridge

The LudgerClean™ CEX cartridge is primed with water.

3 Apply the glycan sample

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

4 Elute the glycans

Bound glycans are washed off the cartridge using water.

5 Post elution workup

Dry down the glycans ready for further analysis.

Time Line for Cleanup

The LudgerClean™ CEX glycan cleanup procedure typically takes around 90 minutes:

Procedure	Time	Elapsed Time (minutes)
Wash cartridges	45 min	45
Apply sample	15 min	60
Elute glycans	30 min	90
Dry glycans	6 hours	-

Sample Preparation

1 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 dry down the sample and reconstitute in water or an aqueous buffer.

Preparation of Cartridges


2 Prime the LudgerClean™ CEX Cartridges

For each sample, prepare a LudgerClean™ CEX cartridge by washing with 10 x 1 ml water
Check the pH of the last 1 mL water wash. After the water washes the eluent should be pH 7.

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 pipette) in order to resume normal flow.
Do not allow the resin to dry out.
Allow each aliquot to flow through the resin bed before the next solution is applied.

Application of Sample

3 Apply the Sample

a. Place the cartridges over a vial that you are going to collect the eluent in
b. Apply each sample to a prepared LudgerClean™ CEX cartridge and allow the solution to flow through the resin bed slowly under gravity.
c. Wash out each vial with 200 µl water and add to the top of each column and allow the solution to flow through the resin bed slowly under gravity.

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

Elution of Glycans

4 Elute the glycans

Elute the glycans off the CEX column by washing with 3 x 0.5 ml water.

The eluted fluid will contain the purified, released glycans.
If the flow through the column is restricted, e.g. by an air gap, then apply a slight pressure to the top of the cartridge (e.g. using a pipette) in order to resume normal flow.
Note that at the stage glycans will be in slightly acidic water after elution from the CEX cartridge.

5 Dry the eluted glycans

If required, and depending on how the glycans will be analysed, the samples should be dried by centrifugal evaporation, then redissolved in a desired volume of water or solvent for further analysis.

Keep the sample temperature <28°C to minimize desialylation.
This step is optional and can be omitted if you are analyzing aliquots by any method that first involves drying (e.g. addition to a MALDI-MS plate or fluorescence labeling).

Sample Storage

6 Store the Glycans Frozen

For long-term storage, store the glycans at -20^{o}C or lower temperature.

The released glycans can be stored frozen either dried or after reconstitution with water.

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.

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